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ALTERNATIVES TO ANIMAL USE IN RESEARCH, TESTING, AND EDUCATION

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Alternatives to Animal Use in Biomedical Research

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Introduction

Factors Affecting Acceptance of Animal Alternatives by the Biomedical Research Community.

Many animal alternatives are well-accepted and widely used in biomedical research. This acceptance and use rests principally on the confidence of individual investigators and their reviewing peers that these methods offer the best available way of obtaining valid, reliable data. After confidence, the next most important item is generally cost, not only the actual expense of purchase and maintenance, but the cost in time and effort. In most biomedical research laboratories graduate students, post-doctoral fellows, or temporary medical residents do a great deal of the actual mechanics of the research; their time with any project is limited. In the best laboratories this results in innovative methods and enlightened use of new technology. Even there, however, conscience may well be the last consideration in the use of alternatives. Many investigators who still find it necessary to use animals do so with some reluctance, but an investigator who cannot produce convincing data and stay within his budget will not long survive in such a competitive field.

Basic Requirements to be Met by both Animal Models and Alternative Methods.

Identification of the most appropriate model is the first hurdle in the planning of any research project. Whether the final decision is the use of a one-cell protozoan, a mammal or a computer program, certain basic needs must be met.

Accuracy of Reproduction/Simulation. Any proposed model must be able to accurately reproduce or simulate the system, disease, or injury to be studied. If possible, it should be available in several variations which may be tested against one another and should have enough background usage for historical comparison. For animal models, data from controls should be compared to data from control animals or tissues of previous studies; significant differences should be justifiable. For non-animal systems, any previously used similar system should be evaluated for demonstrable accuracy of its predictive powers.

Availability. Models should be readily available to multiple investigators in numerous locations at a reasonable cost; they should also be transportable and easily handled (or operated) by investigators.

Longevity. Finally, a good model animal needs to live long enough to reproduce and be studied, while a non-animal model needs to be flexible enough to allow minor alterations in the system or program without compromising the integrity of the whole. Computer programs should always be protected from accidental damage or intentional tampering by a password access system and ample, separately stored back-up.

Several detailed discussions of criteria for animal models and their use in disease research have recently been published (Migaki and Capen, 1984; Gross, 1985).

Figure 4-1

This matrix summarizes 107 responses to a questionnaire distributed to 194 investigators who had used animals for research in 1983-84 at the University of Virginia Medical Center; each faculty member was requested to indicate current and potential usage within his/her research area as follows:

Use of whole animals
Modified use (fewer animals or lower order animals)
Use of animal components
Use of non-living systems (chemical, physical, or mechanical)
Use of computer programs

These replies represent the investigators' perceptions. The matrix should not be interpreted as an all-inclusive summary of the available alternatives in the disciplines listed.

Figure 4-1

POTENTIAL FOR ALTERNATIVES TO ANIMAL USE IN BIOMEDICAL RESEARCH

	Whole Animals	Modified Use	Animal Components	Non-living Systems	Computer Programs
Anatomy	+	+/-	+	+	+/-
Anesthesiology	+	+	+	0	+
Biochemistry	+	+	+	+	+
Biomedical Engineering	+	+/-	+	-	+
Cardiology	+	+/-	+/-	0	+
Cell Biology	+	+	+	0	+/-
Dentistry	+	+	+	0	0
Developmental Biology	+	+	+	-	+
Endocrinology	+	+/-	+	+	+
Genetics	+	+	+	0	+
Gerontology	+	+	+	+/-	0
Histology	+	+	+	0	0
Immunology	+	+/-	+	-	-
Medical Education	+	+	+	+	+
Metabolism	+	-	+	0	0
Microbiology	+	+/-	+/-	+/-	-
Molecular Biology	+	+	+	0	0
Neurology	+	+	+	0	+
Nutrition	+	+	+/-	0	+

Oncology	+	+	+	0	+
Orthopedics	+	-	0	-	0
Pathology	+	+	+	+	+
Pharmacology	+	+/-	+	-	-
Physiology	+	+	+	+	+
Radiology	+	+/-	0	0	-
Surgery	+	+	+	+	+
Teratology	+	+	+	+/-	-
Toxicology	+	+	+	+	+
Virology	+	+	+	+/-	-
Zoology	+	+	+	0	0

KEY + Now in use
+/- Possible
- Doubtful
0 Not Possible

Legend of Terms Used in the Matrix

ANATOMY. The study of the structure of living organisms.

ANESTHESIOLOGY. The study of anesthesia and anesthetics.

BIOCHEMISTRY. The study of the chemistry of living organisms and of their vital processes.

BIOMEDICAL ENGINEERING. The planning, designing, construction, and management of mechanical and electrical devices for medical use.

CARDIOLOGY. The study of the heart and its functions.

CELL BIOLOGY. The study of the structure and function of individual cells or cell types.

DENTISTRY. The study of the teeth and oral cavity, including preventive care, treatment, and restoration.

DEVELOPMENTAL BIOLOGY. The study of the processes of growth and differentiation.

ENDOCRINOLOGY. The study of the endocrine system, the glands of internal secretion.

GENETICS. The study of heredity, including the nature of genetic material and of its control over development.

GERONTOLOGY. The study of the physical processes and medical problems of aging.

HISTOLOGY. The study of the composition and function of tissues.

IMMUNOLOGY. The study of all aspects of the function of the immune system.

MEDICAL EDUCATION. The process of training and developing knowledge and skills in medicine and medical research.

METABOLISM. The study of the physical and chemical processes by which matter is transformed in living organisms.

MICROBIOLOGY. The study of microorganisms.

MOLECULAR BIOLOGY. The study of the behavior of simple and complex molecules within living systems.

NEUROLOGY. The study of the structure and function of the nervous system.

NUTRITION. The study of the intake, transformation, and use of nutrients.

ONCOLOGY. The study of the origins, development, diagnosis, and treatment of tumors.

ORTHOPEDICS. The study of the protection and restoration of the skeletal system.

PATHOLOGY. The study of the changes in tissues and organs during disease.

PHARMACOLOGY. The study of the actions of drugs on living systems.

PHYSIOLOGY. The study of physical structures and chemical processes as they affect function in living systems.

RADIOLOGY. The study of the effects of radiant energy and of its use in the diagnosis and treatment of disease.

SURGERY. The study of the treatment of disease, injury, or deformity by operative methods.

TERATOLOGY. The study of abnormal development and congenital deformities.

TOXICOLOGY. The study of compounds that are poisonous to living systems.

VIROLOGY. The study of viruses and viral diseases.

ZOOLOGY. The study of animal life and animal classification.

Continued, but Modified, Use of Animals in Research

Animal use can be modified in a number of ways: Using fewer animals, reducing the degree of insult, or substituting one organism for another. In the last case, an animal of a "lower" order usually replaces one of a "higher" order, although this is not always true. Commercially produced swine have been used in place of dogs for surgical research and medical education; they proved to be excellent models, were less expensive, and have not aroused the sentiments of the public as had the use of pound dogs (Swindle, 1984).

Methods of Reducing the Number of Animals Used

The importance of the use of parallel, internally controlled designs for experimentation may be one of the first lessons learned by an aspiring student/scientist if the results of his first efforts are rejected for failure to provide for comparable data from treatment groups of animals matched for size, age, sex and dosage. It can be a lesson so painfully learned that he never progresses beyond it to any of a variety of selectively acceptable statistical alternatives. For, while studies with investigator-initiated internal controls support substantially stronger inferences than those without internal controls, there are circumstances in which use of external controls may be possible, preferable or even obligatory (Bailar et al, 1984). For example, in trials of promising new therapeutic agents for terminally ill patients, clinicians may administer treatments to one or a few patients without "matching" them to specific controls, either because controls on other comparable treatments are not currently available or because use of untreated controls in this manner would be inappropriate or unethical.

Common and Historical Controls. Fewer animals may be used in an experiment by sharing a control group with other investigators or by not using a concurrent control group. In each of these instances, it is necessary that all the physical and genetic characteristics of the treatment group(s) be matched to those of the control group and that the conditions under which the data are collected are as precisely duplicated as possible. There are difficulties unique to each of these metnods. Investigators may encounter contraints upon their particular study when sharing controls; for example, if one group needs to extend its studies beyond the time agreed for termination and necropsy of the shared animals, but timing is critical to the results of the other or if the actions of one group adversely affect the other as might happen by inadvertant spreading of a parasite or pathogen. In the case of Historical controls, the difficulty rests in exactly duplicating the conditions of another time; use of such controls must be carefully documented and justified (Geller, 1983).

Animal Sharing. Another method of reducing the number of animals used is to share individual experimental animals or their tissues between groups. While this method may encounter the same types of difficulties described for the sharing of controls, it appears to be gaining in popularity among compatible groups. At the University of Virginia, investigators in endocrinology (Internal Medicine Dept.) and in the molecular genetics of heme synthesis (Biology Dept.) use the pituitaries and livers of the same rats even though the two departments are on opposite sides of the campus (Dierks, 1984). Conversely, using animals maximally in a confined area is a mandatory part of experimental design in the National Aeronautics and Space Administration's animal research program. Typically, protocols call for investigators to combine projects and make efficienct use of one small group of animals (Lewis, 1983). It has been suggested that sharing animals could be a way of reducing animal use in the neurosciences (Hodos, 1983); this would be particularly encouraging, when possible, because this field is not amenable to many of the other alternative methods.

Improved Experimental/Statistical Design. To quote a frequently cited, but critically relevant, comment, "Every time a particle of statistical method is properly used, fewer animals are employed than would otherwise have been necessary" (Russell and Burch, 1959). In the twenty-five years since that was written, progress has been made both in the number of statistical tools available to research and in the training of investigators in the use of these tools. Unfortunately, training still lags behind availability; insufficient information for critical evaluation and inappropriate statistical analyses appear frequently in the literature, particularly in the use of the t-test where analysis of variance is the appropriate measure (Geller, 1983)[1]. Such observations are not esoteric since poor summarization and statistical usage may reflect poor experimental design, calling into question the results of an investigation and leading to otherwise unnecessary repetition. The Harvard Study Group on Statistics in the Biomedical Sciences is pursuing ways to improve statistical practice and reporting (Emerson and Colditz, 1984).

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[1] An analysis of variance simultaneously tests two or more parameters of treatment groups for indication of significant difference; when the test statistic falls in the rejection region, the researcher can be reasonably sure that a real difference exists between treatments. The t-test estimates difference between the mean values of one parameter of two treatments. It is a powerful measure of significance when the number of comparisons is small, but is subject to an increasingly large potential for error as the number of parameters enlarges; using multiple t-tests increases the risk of finding a significant difference between treatments where there is none.

Serial sacrifice, crossover, and group sequential testing are three types of experimental designs which might be applied to reduce animal use in laboratory research (Geller, 1983). In serial sacrifice, animals with induced illness are randomly selected for sacrifice and examination for the occurrence and progress of disease or damage over time. Such studies (as in radiation oncology, Berlin et al, 1979) have the advantages of cutting short the time some animals must spend in the diseased/distressed state and of providing information about changes within the animal other than those observed when it is allowed to die without further interference. The primary disadvantage of this method is that survival information is compromised, therefore, the data resulting cannot be compared to other studies in which survival serves as an end point.

A crossover design may be appropriate for studies in which effects are expected to be short-term. Each animal serves as its own control by first receiving either a drug or a placebo, then being allowed a period of recovery, and finally, receiving the reverse of its first treatment. Such a design can be highly useful in laboratory and clinical testing, but must be used judiciously; if there should be any unexpected long-term effects the entire test is invalidated and would need to be repeated as two separate tests.

In the group sequential design, treatment groups are compared to each other in stages. For example, if two groups are given the same dosage of two different drugs, experimentation at higher dosages is undertaken only if there is no statistically significant difference between the responses of the two groups; the sooner a difference between groups is observed, the fewer the number of trials run. Both the crossover and the group sequential designs have potential applications in comparative studies in anesthesiology, endocrinology, nutrition, pharmacology, radiology, teratology, and toxicology.

The two most commonly mentioned methods of reducing the numbers of animals used are fewer trials and smaller treatment groups. Both of these methods have particular application within the pharmaceutical industry and in hazardous materials testing; efforts in these areas are discussed in chapter 00. In the field of toxicology it has been suggested that exposure of large groups of animals be replaced by exposure of fewer animals to be studied intensely over long periods of time, an approach said to bear more directly on realistic assessment of human risk (Weiss et al, 1983). Within the biomedical research community a frequently heard complaint is that too few animals to yield useful estimates are likely to be included in each treatment group, particularly in fields such as radiology (Herbert, 1984). Problems of this nature generally grow out of the extreme economic pressures being applied to investigators to control animal costs. Well-established techniques such as saturation analyses, particularly radioimmunoassays, have radically reduced the number of animals used for any one procedure, but may have resulted in little or no reduction in overall use since they have made previously difficult analyses more accessible to a much greater number of investigators. A more encouraging view of future reductions is obtained with the realization that, as many of the other alternatives such as in vitro culture and computer simulation are validated and gain wider acceptance, final confirmation using whole animals will require both fewer trials and smaller treatment groups (Attinger, 1984).

Methods of Reducing the Degree of Insult

Anesthesia. Until relatively recently the probability of a research animal receiving the correct amount or type of anesthetic was directly dependent upon the willingness of the individual investigator to accept, reject, or improve upon the information he had from previous research. The advent of facility veterinarians and animal care committees with overseeing authority has resulted in the recognition of the experimental animal as a veterinary patient entitled to protection from as much pain and distress as is possible while still maintaining the integrity of worthwhile research.

Anesthetics may be classified as topical, local or general according to the breadth of their effect. Topical anesthetics have limited use in animal research, primarily as components of ointments applied to minor injuries. Local anesthetics are used for many minor surgical procedures and one, tetracaine, has been recognized by the Consumer Product Safety Commission as suitable to be used in conjunction with eye irritancy tests (Rowan, 1984). Investigators using local anesthetics must be prepared to provide post-procedure care, however, because anesthetized surfaces are particularly liable to accidental and self-inflicted damage (Paton, 1984). General anesthesia, injectable or inhaled, is widely used in biomedical research. Soma (1983) has written an excellent summary of all the major types of general anesthesia available to animal research including species limitations and variations. Paralytic agents are not acceptable as substitutes in procedures requiring general anesthesia, i.e., those involving major or prolonged internal or external trauma. Such agents do not prevent the perception of pain, only its outward expression. Tranquilizers are useful in reducing stress or resistance to confinement, and conscious sedation has been offered as an alternative to general anesthesia for certain dental procedures (Bennett, 1984); these agents are not, however, methods of pain control.

Terminal anesthesia or death as an alternative has become the method of choice following major organ surgery on animals as a part of medical education, even though it might be argued that observation of the healing process should logically constitute a part of surgical training (Wright, 1984). Even in such fields as research cardiology where there has long been resistance to terminal experiments from investigators desiring to fully evaluate post-surgical effects, methods which allow maximum data gathering before death under anesthesia are now appearing (Bloom, 1984). Other types of experiments are terminal under anesthesia due to their special circumstances; one example of this type is in vivo organ perfusion wherein data for endocrinological, metabolic, pharmacologic, or physiological research can be gathered from the functioning organ which is then perfused with a preservative solution and removed for histological examination (Mather, 1983).

Instrumentation. The use of small samples for analysis by mass spectrometry (Meuzelaar et ai, 1984) and by gas or liquid chromatography (Vestal, 1984; Gross and Rempel, 1984) continues to be a favorite example of minimally invasive technology. Even a brief survey of recent clinical and research literature indicates that refinement of established techniques plus much new instrumentation are available for the benefit of man and animal in research, diagnosis, and treatment (McLafferty, 1984). In vivo measurements using fiber optics now provide miniaturized spectrophotometric analysis from within the ducts and blood vessels, determine blood velocity, measure temperature changes, monitor intracranial and intracardiac pressure, measure fluorescent marker molecules in tumors, measure pH, and even determine glucose concentration (Peterson and Vurek, 1984). These and other present and potential uses of fiber optics offer great promise: They can be inserted into vessels and ducts via small catheters with little discomfort and into the abdominal cavity using local anesthesia (laparoscopy); they can be used repeatedly within the same animal to obtain serial samples without permanent damage; and the breadth of their potential applications in biochemistry, cardiology, endocrinology, immunology, metabolism, neurology, oncology, physiology, and other areas of research is limited only by the ingenuity of the investigator. Other minimally invasive techniques include immunoscintigraphy (a diagnostic technique in which a two-dimensional picture of a bodily radiation source is obtained), amniocentesis for the early detection of genetic diseases, teratological events and fetal distress, and laser surgery within the eye (Ewald and Gregg, 1983). A newer application of the laser in oncology involves its ability to initiate a lethal photochemical reaction in cancerous tissue during photoradiation therapy (Christensen, 1984). It is also being used to make sensitive chemical analyses (Zare, 1984). The production of target-specific monoclonal antibodies has improved the ability of external radioimaging techniques to locate tumors and to identify certain noncancerous diseases; radiolabeled antibodies attach to the target tissue which can then be visualized via scintigraphy (East et al, 1984).

Many of the apparently new non-invasive techniques are actually adapted, miniaturized, or computerized versions of older methods. One such example is a small inflatable tail cuff used to measure blood pressure in a rat's tail during hypertension studies (Zandberg, 1984). In other studies, urine is used in some specialized method: In physiological research, electrical impedence measures canine urinary output (Abbey et al, 1977) and in biochemical studies, the presence of certain urinary proteins forecasts potential for visual or kidney failure in diabetes (THE WASHINGTON POST, 1984). Tandem mass spectrometry is being used for breath analysis to screen for diabetes, cirrhosis, renal disease, and ovulation. Many diseases remain to be examined, but there is potential for use of this technique in toxicology, nutrition, metabolic diseases, endocrinology, anesthesia, physiology, and pathology (Manolis, 1983). A technique developed for the determination of the quality of agricultural crops (Norris, 1978) and percent of fat in ground beef (Massie, 1976) uses amplified, digitized, computer corrected diffuse reflectance spectrophotometry in the near-infrared(NIR) region. NIR technology, which involves simply placing an appropriate sensor on the surface of the skin, has replaced the slice-technique previously used to determine the depth of fat by inserting a ruler into a cut made through the dorsal fat on hogs being readied for market and has been or is being adapted for use in oncological, physiological, and nutritional studies (Hruschka, 1984). Other rapidly expanding non-invasive techniques include: Ultrasonography, which is being used in cardiology to locate vessels (Metz et al, 1984), to determine blood flow velocity (Sainz, 1977) and for detection of early atherosclerosis (Johnston, 1977); and nuclear magnetic resonance (NMR) which has recently been used in gerontological research to examine the energetics of skeletal muscle (Taylor, 1984), for clinical diagnosis of metabolic disorders (Budlinger and Lauterbur, 1984), and to provide details of molecular structure and dynamics in liquids and solids (Maciel, 1984).

In the past decade practically every piece of instrumentation in biomedical laboratories has been adapted to handle "micro" samples or has been replaced by new microtechnology. It is impossible to separate into cause or result whether ever smaller samples have demanded ever more sophisticated instrumentation or whether such instrumentation has allowed the use of smaller samples. In reproductive physiology a 1.0 microliter sample of rat epididymal fluid collected by micropuncture can be used to examine sperm motility, determine total protein, undergo microelectrophoresis, and determine androgen binding protein activity (Turner et al, 1979); with a combination of biochemistry and molecular genetics, electrophysiological techniques are being used to explore the possibility of recording the opening and closing of single membrane channels (Iversen, 1984). To study leukemias, other blood diseases, and inborn errors in metabolism a method for measuring the enzyme kinetics within a single neutrophil has been developed (Markovic et al, 1983); and a device is available that will dispense a 1.0 microliter sample as 1000 equal aliquots (1 nanoliter each) for use in biochemical enzyme research or for clinical samples such as cerebrospinal fluid from infants (Hieftje, 1983). These are but a few examples of the many new tools and methods available to biomedical research.

Use of Lower Mammals

National Trends in Animal Use.

Data presented in other Chapters of this assessment demonstrate the current trend toward use of smaller animals in federally funded research. However, since neuroscience has often been castigated as one of the disciplines making unrestricted use of a pet animal, the cat, a recent review is particularly enlightening. In comparing animal use in papers published in BRAIN RESEARCH in 1971 and 1981, Hodos (1983) found that there had been increased use of rodents (3-fold), invertebrates and tissue culture, decreased use of cats (7-fold), monkeys, amphibians and hoofed mammals, and that use of rabbits, fish, Humans and dogs had remained about the same.

"Custom Designed" Rodents. One of the principle reasons for the increased use of rodents in all areas of biomedical research has been the availability of genetically homogeneous and/or pathogen-free strains. However, for some studies, a further degree of genetic definition is needed; these require that the research animal carry some specific genetic traits that are suited to the objectives of the research. Because of their high reproductive potential, rodents are ideal for this type of "custom designing" and extensive use is being made of these animals in a variety of disciplines (Jonas, 1984). Oncology and immunology are two of the more familiar areas of use (Held et al, 1984). Pharmacological research using an ethanol-preferring strain of rats has prepared the way for exploration of the genetics of alcoholism (Waller et al, 1984). The use of the male Lewis rat, an animal which rapidly acquires testicular lesions and antibodies to sperm after vasectomy, has been proposed for a study in the reversal of vasectomy (vasovasostomy); this research could answer questions of human concern in anatomy, physiology, immunology, endocrinology and reconstructive surgery (Howards, 1984).

Moving from Larger to Smaller. In addition to any humane considerations, there are at least three driving forces in the movement toward greater use of the smaller mammals. One is the increasing availability of comparative data; as more information on their physiology, biochemistry, and endocrinology accumulates and is demonstrated to be like or unlike the human's, then more use can be made of them which, in turn, generates more information in an ever-widening circle. Studies in comparative neuroscience are said to be one of the most rapidly expanding fields with relevance to physiology, biochemistry, pharmacology, developmental biology, and zoology (Bullock, 1984); some brain components have been found to be remarkably similar between vertebrate species (Field et al, 1984).

As has been discussed previously, economics plays a large part in the selection of how many and what kind of animals will be used in some research. An investigator following up on previous work will generally begin with the species already in use and only move to something smaller if money gets tighter or if a better model is clearly demonstrated. Investigators starting into newer areas are much more likely to seek the advice of a facility veterinarian as to which species best "fits" his needs and to begin with the smallest acceptable animal.

Use of Nonmammalian Vertebrates

Birds. Chickens and their embryos have played and continue to play a large role in the study of and in the instruction of developmental biology, endocrinology, histology, and zoology. Other current uses are in molecular biology in which enbryonic chicken brain tissue is being cultured to study the neural cell adhesion molecule (Sorkin et al, 1984) and biochemistry in which the embryonic chicken liver is being used to study the acquisition of hormone responsiveness during embryogenensis (Elbrecht et al, 1984). In cardiology research turkeys with inherited Turkey Round Heart Disease serve as models of cardiomyophathy (Jankus et al, 1984) and turkey erythrocytes are fused with amphibian erythrocytes (*Xenopus laevis*) to study receptors which mediate the physiological functions in heart, smooth muscle and other tissues (Strulovici et al, 1984).

Amphibians and Reptiles. Frogs have a long history in research and teaching, particularly in anatomy, biochemistry, developmental biology, physiology, and zoology. They continue to be widely used in those disciplines and , additionally, are currently being used by NASA in radiology studies (Lewis, 1983) and in dental research on digital transplants to augment tooth and jaw regeneration (Howes and Eakers, 1984). The newt, *Triturus*, is capable of regenerating its limbs, eye lens, tail, and spinal cord and is being studied by developmental biologists with the expectation that in understanding the mechanisms, regeneration in humans may ultimately be possible (Hay, 1984). Turtles are also used in biomedical research by physiologists and bioengineers: a new pathway associated with the retinal mechanisms subserving color vision was recently elucidated using the cones of the turtle retina (Normann et al, 1984).

Fish. In general, fish are used in research to a lesser degree than other vertebrates considering that there are over 30,000 species and that they are relatively simple to care for. It has been suggested that they would make excellent subjects for nutritional research since many are known to show specific vitamin deficiency symptoms (Waddell and Desai, 1981). Physiologists have used the goldfish to study the implications of myelin sheath resistances in demyelinating diseases (Funch and Faber, 1984). Rainbow trout embryos are being used in oncology research through exposure to various carcinogens (Hendricks et al, 1984). And, in recent years there has been a great deal of interest in a very specialized feature of some fishes, the electric organ, a tissue exceptionally rich in a single class of cholinergic synapse. Biochemists, geneticists, and molecular biologists working with this material have determined that the structure of the acetylcholine receptor protein is remarkably like that of the human (Changeux et al, 1984).

Use of Non-Vertebrates

Microorganisms. From their origins within the medical disciplines of bacteriology, pathology, and virology, the study and use of microorganisms have branched out to influence practically every area of biomedical research. The following examples are intended only to indicate the breadth of their applications: *Salmonella typhimurium* - bacteria used in the Ames mutagenicity /carcinogenicity test and in mechanistic studies in genetics (Levin et al, 1984); *Escherica coli* - bacteria used by developmental biologists to derive theories of gene control (Hay, 1984) and by molecular biologists in recombinant DNA research (Gall, 1984); *Streptococcus mutans* - bacteria used in dental research on the metabolic activity of plaque (Hayes, 1984); *Bacillus subtilis* (bacteria) spores, *Artemia salina* (brine shrimp) eggs, *Nicotaiana tabacum* (alkaloid producing plant) seeds, *Arabidopsis thaliana* (plant) seeds, and *Sordaria fimicula* (fungi) ascospores - all incorporated into NASA's biostack (monolayers of biological test organisms sandwiched between thin foils of different types of nuclear track detectors) radiology experiments inside Spacelab I (Bucker et al, 1984); *Tetrahymena pyriformis* - a ciliate protozoan being used to study the effects of anesthetics on metabolism (Conklin and Lau, 1984); *Caenorhabditis elegans* - a one millimeter roundworm whose complete cell lineage has been traced by developmental biologists offering an unprecedented opportunity for the study of individual living cells to a variety of disciplines, but particularly to gerontology (Sulston et al, 1983); and a host of microscopic protozoans, metazoans, rotifers, and nematodes are being used to investigate the physiology and biochemistry of photoreception and vision (Ali, 1984), as is *Chlamydomonas*, a single-celled green alga (Rawls, 1984).

Marine Invertebrates. With a few notable exceptions, the marine invertebrates are used less than might be expected considering the ease with which many of them are maintained in laboratory aquaria (Gross, 1985). It has been suggested that the reasons for this are the lack of opportunity by medical scientists to learn marine biology and by marine biologists to learn pathology (Smith, 1979). One exception to this situation led to the development of the *Limulus* amoebocyte lysate test which partially replaced the use of the rabbit for pyrogen testing (Smyth, 1978). Another study uses marine sponges as models for inflammation (Weissmann, 1985). However, the most notable current exceptions are in neurology where the coelenterates, including the hydroids, corals, anemones, and jellyfish, have altered our understanding of primitive nervous system biochemistry, the lobsters and squids have contributed to our knowledge of brain anatomy and physiology, and the grazing snail and crayfish have broadened our understanding of cell biology (Bullock, 1984).

Terrestrial Invertebrates. Neurology has also been one of the principle users of the land invertebrates: flies, bees, earthworms and leeches are employed in various anatomy, physiology, and biochemistry experiments (Bullock, 1984); ants and bees are also used in vision research (Ali, 1984); the tobacco hornworm moth and the aphinx moth are serving as "model" mosquitoes in sensory studies directed toware control of insect vectors of disease (Hildebrand, 1985). Fruit flies, best known for their participation in genetic studies (Struhl, 1985), have been proposed as possible models for screening tests in teratology; embryonic cells placed in culture just after gastrulation are sensitive to a number of known teratogens (Buzin and Bournais-Vardiabasis, 1984). Age-related metabolic changes in other insects are being investigated for possible use in gerontology research

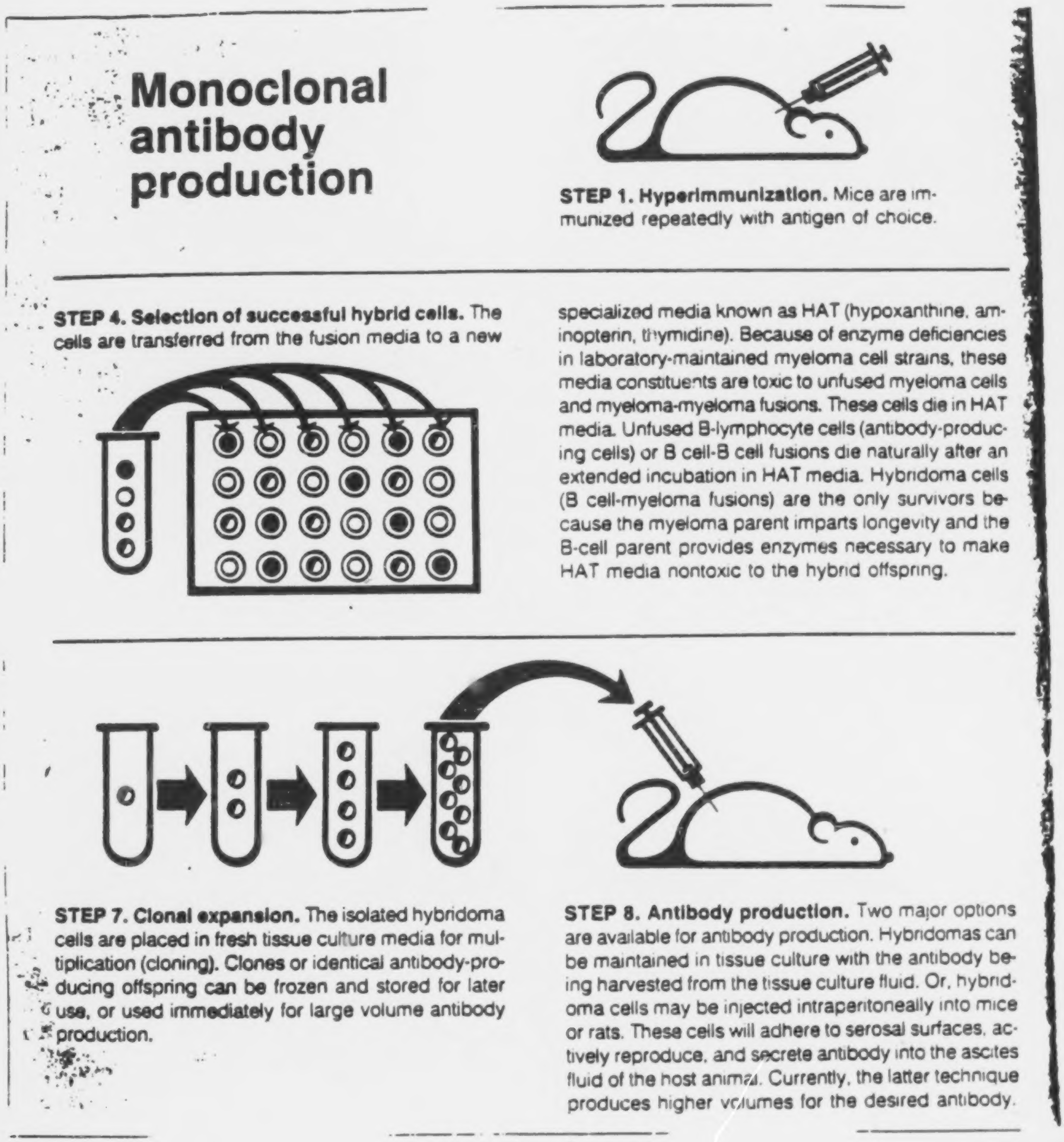
(Sharma et al, 1984). Insects are also being used as models in endocrinology and toxicology (Hammock, 1985).

Use of Living Components in Research

In Vitro Culture Systems

Materials and Methods. In vitro culture systems can be as simple as one culture dish containing a single embryo or as complicated as multiple chambers containing millions of cells destined to participate in fusion and cloning for the production of monoclonal antibodies (Fig. 4-2). However, it would be incorrect to assume that the successful culture of one of these was easier to attain than that of the other. Except for the bacteria and viruses, keeping cultures alive for more than a few hours was more an art than a science until the 1950s when investigators began to gain a better understanding of the requirements of cells and the addition of antibiotics to culture systems extended their viability significantly by controlling bacterial contamination.

The specific conditions which best support the maintenance, growth and/or differentiation of each type of culture must be determined and proven before any useful information can be gathered. Some of the general requirements of culture systems are combinations of the proper gas atmosphere, humidity, temperature, pH and nutrients; others may also have specific light, motion, pressure, and physical or chemical support requirements. Table 4-1 lists some of the components now in use in



Monoclonal antibody production

STEP 1. Hyperimmunization. Mice are immunized repeatedly with antigen of choice.

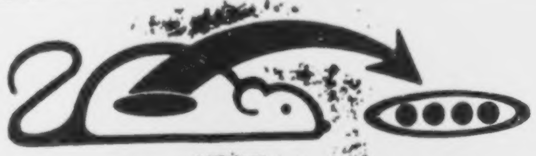
STEP 4. Selection of successful hybrid cells. The cells are transferred from the fusion media to a new specialized media known as HAT (hypoxanthine, aminopterin, thymidine). Because of enzyme deficiencies in laboratory-maintained myeloma cell strains, these media constituents are toxic to unfused myeloma cells and myeloma-myeloma fusions. These cells die in HAT media. Unfused B-lymphocyte cells (antibody-producing cells) or B cell-B cell fusions die naturally after an extended incubation in HAT media. Hybridoma cells (B cell-myeloma fusions) are the only survivors because the myeloma parent imparts longevity and the B-cell parent provides enzymes necessary to make HAT media nontoxic to the hybrid offspring.

STEP 7. Clonal expansion. The isolated hybridoma cells are placed in fresh tissue culture media for multiplication (cloning). Clones or identical antibody-producing offspring can be frozen and stored for later use, or used immediately for large volume antibody production.

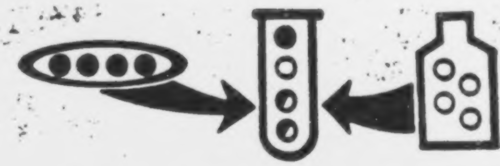
STEP 8. Antibody production. Two major options are available for antibody production. Hybridomas can be maintained in tissue culture with the antibody being harvested from the tissue culture fluid. Or, hybridoma cells may be injected intraperitoneally into mice or rats. These cells will adhere to serosal surfaces, actively reproduce, and secrete antibody into the ascites fluid of the host animal. Currently, the latter technique produces higher volumes for the desired antibody.

Figure 4 - 2

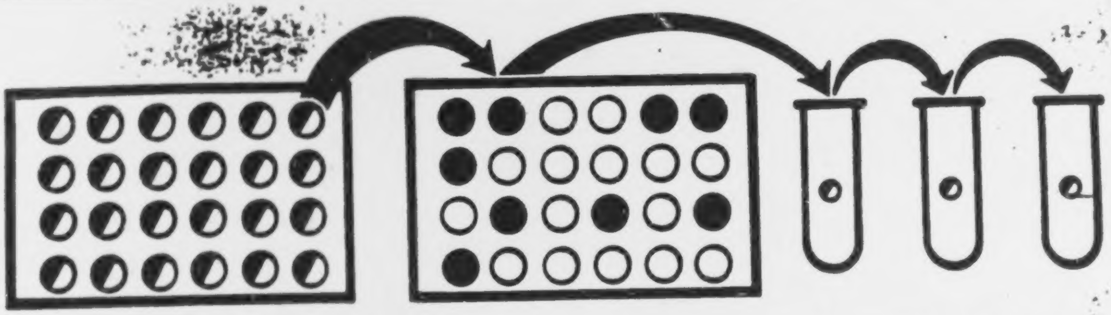
Sherman, D. M., "Genetic Engineering - 3: Monoclonal Antibodies for Veterinary Medicine," VM/SAC 79 (7):959-946, July, 1984.



STEP 2. Collection of antibody-producing lymphocytes. The immunized mouse is euthanatized, its spleen removed and disrupted. Individual antibody-producing lymphocytes (B cells) are collected.



STEP 3. Cell fusion process. B-lymphocytes from Step 2 are incubated with mouse myeloma cells in media containing polyethylene glycol, which promotes cell fusion and hybridoma formation.



STEP 5. Identification of desired antibody-producing hybrids. Surviving hybrids are suspended in fresh media and distributed in microtiter plate wells. After incubation, supernatant fluid from the wells is screened for the presence of the desired antibody, using one of several immunologic techniques. Only a small number of B cells taking part in successful hybrid fusions are producing the antibody for which the mouse was hyperimmunized. So screening is necessary to locate hybridomas producing the desired antibody.

STEP 6. Isolating individual hybridoma cells. The cells in wells identified as producing the correct antibody are subjected to a repeated serial dilution process with distribution of dilute aliquots of cells to new microtiter plate wells. Wells are rescreened repeatedly for the desired antibody. This limiting dilution process is tedious but necessary to ensure that a single parent hybridoma cell is isolated so that subsequent clones produce a highly pure, specific, homogenous antibody.

STEP 9. Antibody collection. To collect the antibody, the animal is euthanatized, and the ascites fluid is aspirated. Depending on the desired use of the harvested antibody, purification may be as simple as centrifugation of the ascites fluid, or may involve more complicated immunologic purification techniques.

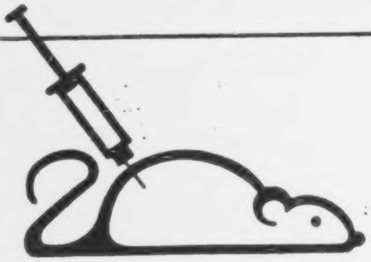


Figure taken from pages 960 and 961

Methods of experiment not involving whole animals

Isolated perfused organs using:	Isolated tissue or tissue sample using:	Isolated single cell using:	Subcellular constituents using:
Liver	Striated muscle	Fat cells	Nuclei
Muscle	Electroplax	Liver cells	Mitochondria
Heart	Iris	Neurones	Microsomes
Lung	Trachea	Glia	Lysosomes
Adrenal	Bronchi	Muscle	Synaptosomes
Intestine	Lung	Smooth muscle	Cell membranes
Skin	Intestine	Red cells	Actin/myosin of muscle
Spleen	Spleen	Leucocytes	
Kidney	Uterus	Platelets	
	Seminal vesicle	Mast cells	
	Vas deferens		
	Bladder		
	Salivary gland		
	Fat pads		
	Liver slice		

Table 4 - 1

Paton, W., *Man and Mouse* (New York: Oxford University Press, 1984)

Table taken from page 104.

biomedical research. Cell cultures taken directly from these living tissues are called primary cultures, while those that are derived from primary cultures are called secondary cultures; under the proper conditions, many of these can be subcultured for months or frozen in liquid nitrogen for years without loss of their unique, differentiated properties. The fact that secondary cultures of human fibroblasts have limited replicative lifespans is being used in gerontological investigations of cellular and molecular aging (Goldstein, 1985). However, the ability to maintain many continuous cell lines has opened the floodgates of experimentation and made the new technologies accessible to all the disciplines of biomedical research. Other advantages include ease of transport from one laboratory or country to another, the ability to culture both normal and abnormal tissue for comparison and testing, the use of human cells to eliminate species variation, and the ability to expose cultures directly to testing agents at specific concentrations for precise time periods. Disadvantages include the changes in structure or function observed in some cultures and the fact that isolated systems give isolated results which may be totally unrelated to results obtained from the integrated systems of whole animals.

Vertebrates and Invertebrates. In addition to their obvious uses in the investigation of basic developmental biology (Sulston et al, 1983; Pederson, 1985), mammalian embryos have been cultured in vitro to examine the effects of hormones (Cline et al, 1977) and of teratogens (Yanai, 1984). The transfer of a growth-hormone gene from a rat into a mouse embryo resulted in a mouse that grew much faster and much larger than normal and stimulated much interest in potential commercial applications within the livestock industry (Gogerty, 1984). Embryos of Caenohabditis elegans, a small nematode worm, and ascidians, primitive vertebrates which form larvan tadpoles, are being used to study developmental phenomena (Wood, 1985; Sternberg, 1985; Jeffrey, 1985).

Unless one considers a tooth to be an organ, whole organs are not generally amenable to true in vitro culture; their size and complexity make it impossible for them to receive sufficient nourishment for normal function without external support. Whole organs continue to be heavily relied upon in many types of anatomy, histology and pathology research because of their suitability for the examination of relationships between cells and tissues. Cryostat sections, (thin slices of frozen tissue cut with a microtome) across organs are being maintained in vitro for oncology studies into organ-specific adhesion of metastatic tumor cells; this method closely reflects in vivo events and , therefore, has potential for eventual reduced use of whole animals in a very active research area (Netland and Zetter, 1984). The use of teeth refers to dental research into the prevention of contraction gaps wherein extracted teeth are maintained in vitro following filling (Jorgensen and Hisamitsu, 1984).

In tissue culture, explanted pieces of a living organism are maintained with their various cell types arranged as they were in the original organism and with their differentiated functions intact. Such cultures are both "better" and "worse" than cultures of a single cell type. They are better in that they offer the opportunity of observing the effects of manipulation in a more natural environment, allowing the different cell types to interact as they would in vivo; but, they are worse in that they are much more difficult to maintain. Tissue culture is being successfully employed in many disciplines. In neurology, the use of embryonic rat mesencephalon explants to examine the destruction of dopamine neurons has replaced the use of primates (Mytilineou and Cohen, 1984); the monkey had served as the best model for the study of degenerative effects observed in humans prior to this investigation because adult rats, cats, and guinea pigs had been shown to be resistant. Metabolic studies of an experimental antiarthritis agent use everted hamster and rat intestinal wall (Tepperman et al, 1984); and physiological experiments examine the dynamics of secretion within explanted mouse epididymis (Flickinger, 1984). Although tissue culture experiments require the sacrifice of an animal, they can truly be viewed as alternatives since numerous sections of adjoining tissue can be explanted and compared using two or more treatments that would otherwise have to be administered to a number of individual animals.

Although cell culture is not a new technique (see Table 4-2), developments and applications during the past decade have come so rapidly as to create whole new research institutions and industries; the potential benefits touch every discipline of biomedical research and clinical practice. Eggs and sperm from many species have been used by endocrinologists, physiologists, and biochemists to study the mechanisms involved in fertilization and early development (Dukelow, 1984; Naz et al, 1984; Oliphant et al, 1984); a hamster ovary cell line and its mutants are being used to explore the biochemistry of a membrane-associated protein essential to all animal cell function (Sahagian and Neufeld, 1983);

Table 1. *Some landmarks in the development of animal cell biotechnology*

1907	Growth of nerve tissue *in vitro* (Ross Harrison)
1916	Trypsinisation to produce single cells (Russ and Jones)
1920-1940	Descriptive work on growth, nutrition, division, differentiation and tissue organisation
1940	Antibiotics — increased longevity of cultures
1948	First cloned continuous cell line — L cell
1950's	Defined (synthetic media)
1943	First continuous human cell line — HeLa
1954	Polio vaccine in primary cells
1962	WI-38 cell line — normal (diploid) human cell
1965	Somatic cell hybridisation — inter-specific heterokaryons
1970's	Human diploid cell vaccines Discovery of specific growth factors Development of large scale unit process culture techniques
1975	Hybridomas — production of pure antibodies
1979	Recombinant DNA hybridisation

Table 4 - 2

Griffiths, B., "Animal Cell Cultures in Medicine," The Lord Dowding Fund 21:5-12, Spring,1984.
Table taken from page 5

in oncology research, human interferon derived from bacterial recombinant DNA induces human monocyte transformation similar to that observed during infection, cancer and rheumatoid arthritis, providing clues to possible pathologic relationships (Weinberg et al, 1984); steriod metabolism is being studied using cultured rat epididymal cells (Brown and Amann, 1984); a monkey kidney cell line was employed to demonstrate the metabolic effects of several general anesthetics (Brabec et al, 1984); geneticists are developing an in vitro method of studying heme gene expression (Dierks, 1984); surgical research into the use of autologous cultured human epithelium for permanent coverage of large burn wounds has moved from the laboratory into clinical trials (Gallico et al, 1984). In studies on antibody synthesis and response in vitro 200-400 observations can be made with the cells from a single mouse (Mosier, 1985). The use of monoclonal antibodies to human cells is an active area of research in immunological studies of T-cell proliferation (Rheinherz, 1985) and in endocrinology (Ciocca and Dufau, 1984). A full detailing of the current research being conducted with monoclonal antibodies from cloned cells would fill a large text. Table 4-3 lists some of the diagnostic potentials of monoclonal antibodies being explored by publically funded research and by private industry.

Diagnostic Potentials of Monoclonal Antibodies

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1. Characterization of malignant and benign tumors
2. Identification of autoimmune antibodies in rheumatoid arthritis, systemic lupus erythematosis, myasthenia gravis and other autoimmune diseases
3. Identification and quantification of serum proteins, hormones and their cell surface receptors
4. Monitoring or therapeutic drugs and identification of novel therapeutic drugs
5. Rapid diagnosis of bacterial, viral, mycotic and parasitic diseases
6. Monitoring and identification of lymphoid and hematopoietic cells in disease states
7. Pregnancy testing

Table 4 - 3

Biologists have developed techniques for the controlled disruption of cells which with careful application can leave many organelles intact or allow the harvesting of selected intercellular membranes. These fractions have proven to be invaluable in the search for information at the molecular level. Microsomal membrane fractions from rat and human liver have been used in comparative anesthesia research (Bradshaw and Ivanetich, 1984); in endocrinology, human placental and ovarian microsomes were used to demonstrate inhibition of steriod hormone synthesis by plant flavanoids (Kellis and Vickery, 1984); longitudinal studies of human lymphocytes have shown degenerative changes during gerontology research (Beregi and Regius, 1984); in dentistry, proteins purified from unerupted fetal buds were shown to be inhibitory toward seeded enamel growth (Doi et al, 1984); in virology, infected cell nuclei isolated from a hamster cell line were used to study influenza virion RNA replication (Beaton and Krug, 1984); and in metabolic studies, the inhibition of chick embryo derived collagen fibril formation by glucose suggests a direct relationship between excess glucose and poor wound healing in diabetes mellitus (Lien et al, 1984). Some of the most interesting uses of subcellular fractions have involved the bringing together of mixed species systems in biochemical studies of protein transport across intercellular membranes. For example, in one part of an ongoing study of protein translocation, investigators used dog pancreatic microsomes, bovine pituitary and rabbit reticulocyte messenger RNA, bacterial nuclease, and a wheat germ cell-free system to elucidate the structure of the essential and controversial signal recognition particle (Walter and Blobel, 1982).

Microorganisms and Plants. A number of the important uses of microorganisms in vitro have already been mentioned in the section on non-vertebrates. At least two of these warrant further discussion: The Ames mutagenicity/carcinogenicity test (McCann et al, 1975) and recombinant DNA technology (Alberts et al, 1983). The Ames test, used primarily in toxicology, employs a mutant strain of bacteria, *Salmonella typhimurium*, which reverts to its wild type in the presence of a mutagenic substance. Chemicals which are mutagenic in these types of assays are said to have an 80% probability of also being carcinogenic in rodent in vivo tests (Brusick, 1983). Recombinant DNA technology has so changed our understanding and expectations concerning regulation of gene expression that, as with monoclonal antibody technology, we can see no end to the potential applications. DNA is now one of the easiest macromolecules in the cell to analyze and it can be readily cloned by integration into a rapidly replicating plasmid or viral genetic element for amplification by bacterial or yeast cells. In this way almost any desirable protein, such as hemoglobin or insulin, can be made in large quantities by bacteria or yeast. One recent application of this technology in oncology is the adoptive immunotherapy of established pulmonary metastases wherein human interleukin-2 produced in bacteria (Escherichia coli) significantly reduced the number of cancerous nodules in mice; clinical trials in humans are underway (Mule et al, 1984).

Although there is some interest in the potential use of plant cells in toxicology and oncology research (Smyth, 1978), the use of whole cells from plants is obstructed by their very rigid cell wall structure as compared to the relatively fluid animal cell membrane. This prevents their use in many disciplines where intimate cell surface contact and/or transmembrane communication is essential.

Once removed from the cell, comparable organelles from plants and animals (including microorganisms) are essentially indistinguishable in both appearance and function. In studies having potentially broad applications in endocrinology and immunology, yeasts have been found to contain active steroid hormone systems

(Kolata, 1984a). Yeast is also being used by cell biologists in research into the import of proteins into mitochondria, organelles that are essentially the same whatever their source (Maccecchini et al, 1979). An extensive literature in cell biology, genetics, molecular biology, and virology supports the use of subcellular fractions from plants and animals, separately or together, for research into basic molecular mechanisms (see Walter and Blobel above).

Appropriate Uses of Culture Systems. The use of in vitro culture is appropriate in any area of research in which it is demonstrably good science and is economically feasible. The most widespread use of in vitro systems in biomedical research is in the study of fundamental properties of cells and of intercellular events. While there is some awareness of the potential clinical and/or commercial applications of their research, most investigators using these techniques view them simply as the best methods of arriving at answers to questions at the cellular and molecular levels. They are acutely aware that they have altered some functions by moving their investigations from an in vivo milieu to an in vitro one, and take great pains to perfect their systems and to qualify and justify their results. Although numbers of animals used per assay have been greatly reduced, in many cases final justification still rests with presentation of supporting evidence from whole animal studies. To a great extent, this kind of evidence is also ultimately required of the better known clinical and toxicological applications of in vitro technology: The Ames test, the Limulus amebocyte lysate or pyrogen test, the McCormack surfactant assay for eye inflammation, and the HeLa cell metabolic inhibition test. Others which do not require animal confirmation include the production of live vaccines (although their safety testing does require animals), histocompatability antigen detection for tissue transplantation using mixed lymphocyte cultures, and the hemagglutination inhibition pregnancy test (Stark and Shopsis, 1983).

Blood

Human Blood and Blood Products. These are used in a variety of ways in biomedical research; several of these have already been mentioned in association with in vitro culture and testing. Another recent investigation in oncology/virology used T-cells cloned from human peripheral blood mononuclear cells in the study of leukemia-lymphoma virus (Mitsuya et al, 1984). Pooled human serum has been employed to investigate the effects of anesthetics on the microbicidal activity of leucocytes (Welch, 1984) and in gerontology studies, the biochemical activities of erythrocytes from young and old individuals were compared (Vomel, 1984). Serum from humans with Myasthenia Gravis is being used in the study of auto-immune disorders (Drachman, 1985). This interesting research thus uses one treatable human disease as a model for similar diseases about which less is known. Blood collected from blood bank donors supplies heparin, serum albumen, and factors VIII and IX for research and clinical use after it is no longer usable as fresh blood for transfusions.

Animal Blood. In research centers animal blood is collected from many species: Large quantities of sterile sheep blood are collected from rotating donors to be used in microbiology and virology research and in clinical diagnosis using blood-agar plates; smaller quantities may be collected as needed from other species for the same purposes; cows and large dogs are often maintained as occasional blood donors for animals being used in cardiology research; such donations do no permanent harm to the donors, anymore than to human donors, but certainly serve to reduce post-surgical trauma and distress for other animals.

Seroepidemiology. This is the use of blood for one purpose that was collected for another. While not new to research, it appears to be gaining in popularity due to its cost effectiveness compared to initiating new blood collections. Samples that were collected ten years ago as part of a hypertension detection study were recently used to answer questions in oncology concerning blood vitamin levels in people who subsequently developed cancer; 10,940 men and women who had participated in the original study were followed for five years; the new study selected participants who got cancer and chose two controls for each matched for age, sex, smoking history, month of blood collection, initial blood pressure, use of antihypertension medication, and, for women, number of children and menopausal status; blood levels of vitamin A, Vitamin E, or carotenoids before diagnosis of cancer showed no significant differences from controls (Kolata, 1984b). Another oncology study on the occurrence of T-cell leukemia-lymphoma virus antibodies in African populations used samples collected for surveys of infectious diseases; a highly sensitive enzyme-linked immunosorbent assay (ELIZA) was used to detect the antibodies in stored serum samples of people from South Africa, Uganda, Ghana, Nigeria, Tunisia, and Egypt; 131 pf 1890 sera tested were positive; occurance was found to be unrelated to race or country of origin (Saxinger et al, 1984).

Organs

Perfusion. At some point in the history of research, investigators have attempted, with varying success, to perfuse every major and minor organ of the animal and human body to gain basic functional information; however, even when the purpose has remained the same, the probability of success has certainly increased due to greatly improved techniques. Several current applications of perfusion include the study of protein synthesis in lactating guinea pig mammary tissue (Mather et al, 1984) and the use of human placentas in toxicology studies with potential for use in oncology and gerontology research (Hodgkinson, 1984).

Transplantation. Although the basic transplantation techniques developed over the past three decades have undergone consistent improvement and refinement, the most difficult research and clinical problems have centered around the post-operative management of organ rejection. Since not all patients needing transplanted organs can depend upon having a relative willing or able to donate a kidney, for example, great dependence has been placed on immunological research into the matching of patient tissues to those of dead or dying donors (Sanfilippo et al, 1984). Other investigations have emphasized the importance of developing drugs such as cyclosporine which suppresses the organ-rejecting immune reaction, but preserves the ability to fight infections (Held and Doherty, 1984). As a result of other types of transplantation research, medical use of animal skin for temporary closure and stabilization in critically burned patients can now be replaced by use of cadaver allografts (Fisher, 1981), and, in gerontology/neurology research, fetal septal tissue has been transplanted into the brains of aged rats with subsequent improvement in learning performance (Gage et al, 1984).

Use of Nonliving Systems in Research

Long before the advent of modern technology into research, scientists were constructing conceptual and mathematical models of physiological events based on their experimental observations (Michaelis and Menten, 1913; Lineweaver and Burk, 1934). Any attempts to apply chemical, mechanical, or computer technology to the question of nonliving alternatives is useless without first gathering together a set of related ideas to explain the functions of a system, a conceptual model, and then forming an equation or formula to account for possible variables, a mathematical model. One must know a great deal about a living system before one can begin to make predictions, let alone draw conclusions, from a nonliving system.

Chemical Systems

Enzymology is one of the areas of biochemistry in which a great deal of experimental information is available. Because of this, biochemists are applying some of the newest instrumentation to the study of enzyme mechanisms in a totally chemical system. Using high-resolution carbon-13 nuclear magnetic resonance (NMR) spectra of enzyme-inhibitor and enzyme-substrate complexes in aqueous solution they can obtain detailed structural and stereochemical data. By combining NMR with cryoenzymology, the use of cryosolvents at sub-zero temperatures, they can even slow down reactions enough to study intermediates which would ordinarily exist for far too short a time to be detected. Investigations which have been restricted to in vivo determinations can now be expanded into a far wider range in vitro (Mackenzie et al, 1984). A physicochemical model system has been developed for dental research which uses a two-chambered diffusion cell. One chamber contains an excess amount of specific protein crystals, the model "lesion", while the other is filled with a chemical solution of artificial "plaque-saliva". Dissolution of the crystals can be studied under varying chemical conditions relevant to a better understanding of the caries process (Chow and Brown, 1984).

Physical/Mechanical Systems

Except for the use of manikins to simulate accident victims in the transportation industry and in trauma centers, the principal use of physical/ mechanical systems is in medical education rather than research. Audio-visual demonstrations and even hands-on experience with devices such as a plastic, computerized dog can only teach that which is already known by someone (Begley et al, 1984). Until some much more innovative technology is available, models of this type will remain outside the realm of biomedical research use. However, it is not inconceivable that future combinations of mechanical, chemical, and electronic technology could provide science with artificial research subjects capable of independent, unanticipated responses.

Epidemiology: Using Existing Data Bases

The use of existing data bases to gain new information and insights in research may be one of our major underused resources, if the paucity of published results is any criterion. One recent study which employed such information concentrated on the relationship between 17 nutrients and the potential for development of hypertensive cardiovascular disease in more than ten thousand individuals from the data base of the National Center for Health Statistics, Health and Nutrition Examination Survey, HANES I, (McCarron et al, 1984a). The results from this study proved to be highly controversial with some of the criticism aimed at the use of the data base (Kolada, 1984c). Other criticisms concentrated on alleged conceptual and statistical problems, but did not disagree with the study's conclusion that there is an inverse relationship between dietary calcium and blood pressure (Feinleib et al, 1984). McCarron has defended his concepts and statistical analyses, stating that review of his procedures and new studies using the HANES

I support his methods and conclusions (McCarron et al, 1984b). All of this controversy may help to explain why greater use has not been made of these types of resources.

Use of Computer Software in Research

In the field of artificial intelligence, the expectations of biomedical scientists have run far ahead of reality (Waldrop, 1984). From DENDRAL, a program on chemical structures in the 1960's, to PUFF, a new program on the interpretation of pulmonary tests, the underlying difficulty has not changed; scientists want software that "reasons", preferably much faster than the human mind can perform; what they have is software that compiles, computes and makes limited comparisons at great speed, but lacks the ability to choose among large numbers of complex variables to reach a conclusion. To use an example from Waldrop (1984), chess has some 10^{120} sequences of legal moves; a general program attempting to "play" the game using all possible moves would never finish. A specialized program, given limited choices, could finish, but it is exactly this limitation of possibilities that the scientist usually wants to avoid.

The principal criticisms of the use of computer programs as alternatives to animals in research have been that much of the basic physiological information needed to make the programs complete is still lacking and that even when the information is available, the fastest machine can handle only a limited number of sequential variables. Neither of these criticisms is unanswerable, given enough time, money and effort. One encouraging point is that, even with these limitations, tHere are a number of useful programs available and many more are under development. Another is that help may be on the way from sources totally removed from biomedical science. A new algorithm has been found which speeds up the solution of linear programming problems at least fifty-fold and which can be applied to problems too large or complex for any current method to handle, those having as many as a million inequalities (Kolata, 1984d).

Chemical Models

Modern quantum chemistry and pharmacology are based on the relationship of chemical structure to activity. This relationship is being used to make preliminary identification of both the toxicity and efficacy of compounds and to tell investigators which differences between compounds are trivial or significant; it is also being used by the National Cancer Institute to screen new compounds for antitumor activity by surveying each molecule within the compound for uniqueness and activity (Holden, 1984).

There are several major methods used to study the relationship of chemical structure to biological activity: Hansch analysis or linear free energy relations (LFER), the Free-Wilson model, and quantum mechanics

(Stuper et al, 1979). LFER uses linear free energy-related parameters as independent variables in an empirical model of drug action; it has applications in pharmacology, biochemistry, cell biology, neurology, molecular biology, toxicology, virology, microbiology, anesthesiology, endocrinology, and oncology. The Free-Wilson additivity model defines activity in terms of the relationships of substituent groups; its primary application has been in pharmacology. Quantum mechanical methods are based largely on the molecular orbital theory of the electronic structure of molecules; they have been used in research in pharmacology, oncology, and neurology.

Simulation of Physiological Functions and Reactions

The usefulness of simulation programs is directly dependent upon how well the system in question is already understood and whether or not it can be defined in mathematical terms (Brady, 1983). The three principal concerns of the investigator must be:

The starting values - to define the experiment

The operating algorithms - to manipulate the values and to generate the simulated data

The data presentation - to properly display the data in graphs, tables, and text

Examples of recent uses of simulation programs in research include neurological studies of impulse propagation along myelinated axons (Funch and Faber, 1984), developmental biology research on the changing shapes of embryonic cells from which mature organs develop (Hilfer and Hilfer, 1983), and initiation of biomedical engineering investigations into malfunctions of instrumented cardiovascular control systems (Attinger, 1984). Simulations have also been used in the past to model cardiovascular function, anesthesia uptake, decompression sickness, drug metabolism and distribution, receptor function, and control of respiration (Paton, 1984).

One development in this field deserving particular attention is the establishment by the National Institutes of Health of the National Biomedical Simulation Resource, a computer facility at Duke University which may be used directly on site or remotely over a telephone data network. Use of this facility is available by application to any project where the results are free to be published in open scientific journals and where no profit is involved. Projects now underway involve research in cardiology, physiology, endocrinology, toxicology, and neurology. Specific simulations include: Regulation of sodium, potassium, and calcium in heart muscle; electrolyte diffusion in heart muscle; propagation of activity in heart muscle; heart volume potentials; mathematical modeling of blood coagulation; regional dose responses in the human and animal lung; ciliary motility; cochlear function in the inner ear; and a molecular model of ion transport in nerves and muscles (Kootsey, 1984).

Compilation, Computation, and Comparison

In addition to those already mentioned, contributions of computers to biomedical research include performance of laborious calculations on experimental data, control of experimental systems so that serial procedures are carried out automatically, information storage and retrieval, and testing of theoretical ideas (Smyth, 1978). Although these are viewed as the more mundane functions, they can be combined with other technology in innovative ways. Investigators and technicians from many disciplines are working together to construct a Human Protein Index from such a combination. When completed, it will be an extremely valuable tool for pathologists, clinicians, toxicologists, geneticists, biochemists, and immunologists (Anderson and Anderson, 1982).

Other Considerations

Humans as Research Subjects

The use of humans as experimental subjects has a lengthy history; in fact, most of our information on traditional remedies has grown through trial and error experimentation on ourselves. Research into pain, sensation, and conscious muscle control has been and continues to be conducted on humans because only humans have the capacity to both follow specific instructions and respond with descriptions of their perceptions. Humans are also the final research subjects for all therapeutics and vaccines marketed for their use; students and employees of large medical centers are frequent participants in such studies. The primary fallacy in some of these types of research as applied to treatment is the placebo effect wherein false "positives" must be ruled out because a certain percentage of human subjects will respond well to any treatment; in that regard, animals can be better subjects since they do not exhibit such effects. One other important criticism leveled at many of these studies is that even when the agent tested is intended primarily for use in the elderly, research subjects are drawn from among the young and healthy; treatment dosages and combinations chosen from these studies may have little relevance to results in the elderly (Greenblatt et al, 1982). Many of the uses of humans and human tissues have already been introduced in other sections of this assessment, particularly in the use of living components. Among those previously cited are human blood and blood products, organs, glands, skin, fetal bones, teeth, and subcellular fractions.

The use of postmortem material from humans has significance in many areas of biomedical research, but particularly in neurology. Investigators studying the unconventional slow virus diseases use brain tissue from humans with Creutzfeldt-Jakob disease and from animals with scrapie (Merz et al, 1984). Postmortem material from schizophrenics has provided evidence for two distinct categories of that disease (Seeman et al, 1984) and temporal lobe structures from Alzheimer patients have revealed specific pathological cellular patterns in the hippocampal formation (Hyman et al, 1984).

Mechanical and electrical hearts, initially tested in animals, are now being attached or implanted in humans with terminal heart conditions, even though these devices are known to be temporary and experimental (THE WASHINGTON POST, 1984).

The genetic factors involved in alcoholism have recently been investigated using the sons of alcoholic fathers. Although the neurophysiological deficits observed in chronic alcoholics have been presumed to result from their disease, studies of their sons indicated the same deficits in them, even when the boys had been adopted into non-alcoholic homes soon after birth (Begleiter, 1984).

Cultured human fetal lung cells have been found to be excellent hosts to support the developmental cycle of a protozoan parasite which causes severe, persistent, life-threatening diarrhea in immunodeficient patients such as those with AIDS. There has been no effective therapy for this illness, so the in vitro system offers an opportunity to study the parasite's behavior, development, and metabolism and provides a potential method for screening therapeutic agents (Current and Haynes, 1984).

In other developments in research related to AIDS, investigators screening persons who had close contact with known victims have isolated AIDS virus in their saliva; few AIDS patients have the virus in their saliva. It is not known yet whether there is some sequential association of these events (Russell, 1984a), but the possibility exists that the disease may be transmitted by saliva (Groopman et al, 1984).

Virologists and oncologists have been very quick to take advantage of new human in vitro culture systems. An embryonal carcinoma cell-line from the stem cells of a teratocarcinoma is being used to study cytomegalovirus replication (Gonczol et al, 1984); lysis of herpes symplex virus (HSV) type 2 is being investigated using human monocytes (Koff et al, 1984) and HSV latency is studied by using isolated human fetal neurons (Wigdahl et al, 1984); colon mucosal epithelial cells have been cultured in vitro and treated with an oncogenic simian DNA virus and a chemical carcinogen which altered a number of their characteristics in a manner suggesting malignant transformation, thus demonstrating the possibility of an additional carcinogenicity testing system (Moyer and Aust, 1984).

While few doubt the relevance of using humans and human tissue for investigations aimed primarily at human treatment, new developments are not without critics and dilemmas. Both the scientific and popular press have focused attention on the ethical and legal issues involved. There has been a request for Congress to establish a national commission for consideration of ethical and social ramifications of new reproductive technology (Russell, 1984b), an expression by some members of congress of a desire to totally ban the already severely limited use of human enbryos or fetuses in medical research (Cohn, 1984), and protésts from animal welfare advocates and concern for consumer reaction about the use of human genetic material in research and food animals (Russell, 1984c). Research institutions conducting studies with human embryos must depend upon funding from commercial sources since federal support for that purpose was banned (Russel, 1984d). Gerontologists investigating Alzheimer's disease are divided among themselves as to the legal and ethical questions involved in obtaining informed consent from patients who are patently imcompetent and for whom there will be no direct benefit from the experiments in which they participate (Kolata, 1984d). Legal questions have also developed from the use of human cell lines concerning the ownership rights of the contributing patient (Culliton, 1984). None of these dilemmas have simple solutions and all are likely to be argued for years in the press, the courts, the scholarly literature, and institutional review boards.

Spontaneously Occurring Models for Disease

One area of research animal use about which there has been less controversy, possibly due to its being less widely discussed, is the use of spontaneous models; these are animals in which a disease occurs naturally and mimics some aspect of human disease. Although diseases which occur as isolated cases may be of some interest in selected instances, by far the most useful spontaneous models are those in which the disease or a susceptibility to the disease is heritable. Conditions which mimic human diseases occur in feral, domesticated and laboratory bred animals, but the well-defined laboratory species are the ones most frequently used.[2] These animals have been invaluable in the study of hypertension, autoimmune diseases, tumor susceptibility, diabetes, and a host of other inherited disorders in practically every discipline of biomedical research. Nearly 900 spontaneous models had been catalogued by 1978 (Hughes and Lang, 1978) and these, plus many others discovered since then, are detailed in several recent publications (Capen et al, 1981; Hegreberg and Leathers, 1982).

The Relationship of Timing to Technology

In biomedical research, technology can be used either instead of or in addition to something else. For example, in many areas of research in which spontaneous models are now used, technologically induced models (subjected to radiation, heat, surgery, drugs, etc.) were used prior to discovery of animals with the naturally occurring diseases. To have waited for the appearance of these animals to pursue research in critical human health areas could be considered grossly negligent. Additionally, without the information gained from the induced models, many of the spontaneous ones might have gone unrecognized. Increased usage of all of the newest technology in in vitro culture, nonliving systems, and computer software has been primarily as additions to the use of whole animals. Very few of these techniques and their instrumentation have been employed for a sufficient length of time for conscientious investigators to rely solely on nonanimal results. Not only must a researcher be concerned with the ultimate effects of his findings at the time of patient trials, but he is also aware that publishing bad data may lead other groups to waste time, money or lives. At best, research can be considered to be in a period of reduced animal use per experiment. With time, technologies can be expected to improve, and their results will become more reliable and acceptable.

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[2] As used here, feral species are wild or free-roaming animals; domesticated are pet or farm animals; and laboratory species are those bred exclusively for research use. Some species, such as rats, may appear in any of these categories and would, therefore, be defined according to their origins.

Biomedical Research Areas with Few, if any, Alternatives.

When queried directly, some investigators in each of the disciplines covered in this assessment have said that there are no alternatives to using animals and in a few disciplines nearly all have said there are none. The fact that this is demonstrably untrue is not due to ignorance, reluctance or insensitivity on their part, but rather to a problem of semantics. Within the biomedical research community all of the methods and technologies explored in this assessment are considered to be adjuncts, not alternatives, to animals. There are clearly areas of research within each discipline which still require the use of whole, adult animals at some point, if only to confirm the results from alternative studies. For example, developmental biology depends heavily upon in vitro studies using cells and subcellular fractions, and computer simulations are just being introduced. However, no matter how much information is gathered or how many theories are proposed, only the use of a whole animal, usually one with a single genetic aberration, can answer the final questions concerning the validity of their experiments and theories. The armadillo is the only immunologically intact animal susceptible to infection by Mycobacterium leprae, the etiologic agent of leprosy, and the only model that reproduces any of the clinical forms of the disease in humans; infected armadillo tissue is also the source of leprosy bacilli used to provide lepromin, a reagent for accessing the prognosis of leprosy in humans·(Delaney, 1984). Goats and pigs have become the animals of choice in wound ballistics research; while high speed photography of gelatin or soap blocks subjected to high velocity projectiles can provide a generalized demonstrattion of cavitation effects (U.S. Dept. of Defense, 1984), only living tissue can show the dynamics of the ballistic effects of bullets of differing velocities (Fig. 4-4) and provide vital information on how best to identify and treat the various types of wounds; knowledge of these phenomena are essential in the training of military surgeons, forensic pathologists, and law-enforcement officials; animals used in these studies are anesthetized prior to being wounded and are terminated while under anesthesia (Swan and Swan, 1984). Most disciplines are still so heavily dependent upon whole animals, that all other technologies are truly adjunct even though they may be well developed and frequently used for selected "pieces" of information. These include anatomy, anesthesiology, biomedical engineering, cardiology, dentistry, endocrinology, gerontology, immunology, medical education, metabolism, neurology, nutrition, oncology, orthopedics, pharmacology, physiology, radiology, surgery, and zoology. Of these, some have few viable options because

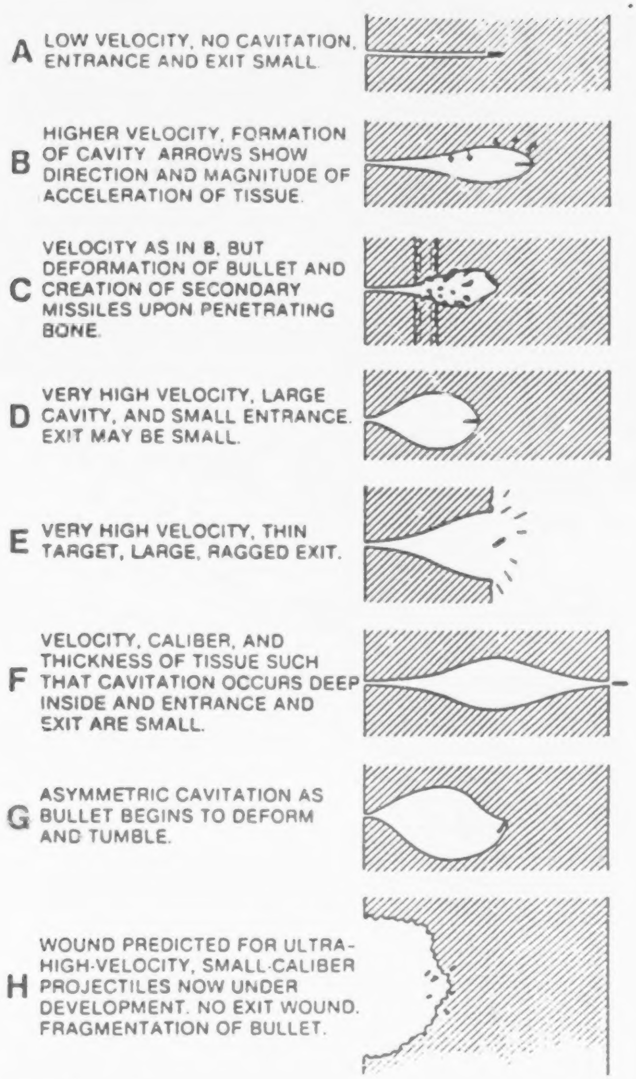
A LOW VELOCITY, NO CAVITATION, ENTRANCE AND EXIT SMALL.
B HIGHER VELOCITY, FORMATION OF CAVITY. ARROWS SHOW DIRECTION AND MAGNITUDE OF ACCELERATION OF TISSUE.
C VELOCITY AS IN B, BUT DEFORMATION OF BULLET AND CREATION OF SECONDARY MISSILES UPON PENETRATING BONE.
D VERY HIGH VELOCITY, LARGE CAVITY, AND SMALL ENTRANCE. EXIT MAY BE SMALL.
E VERY HIGH VELOCITY, THIN TARGET, LARGE, RAGGED EXIT.
F VELOCITY, CALIBER, AND THICKNESS OF TISSUE SUCH THAT CAVITATION OCCURS DEEP INSIDE AND ENTRANCE AND EXIT ARE SMALL.
G ASYMMETRIC CAVITATION AS BULLET BEGINS TO DEFORM AND TUMBLE.
H WOUND PREDICTED FOR ULTRA-HIGH-VELOCITY, SMALL-CALIBER PROJECTILES NOW UNDER DEVELOPMENT. NO EXIT WOUND. FRAGMENTATION OF BULLET.

Figure 4 - 4

Schematic diagram of ballistic effects of bullets of differing velocities upon tissues of varying density and thickness.

they are structurally dependent (anatomy, orthopedics, radiology, surgery), while others require integrated systemic responses for full expression of effects (endocrinology, immunology, metabolism, nutrition, pharmocology).

Current Needs Relevant to the Use of Alternatives

Comprehensive, Accessible Information Systems

Despite a number of successful and attempted national data banks for scientists, there is still a need for better communication between information centers and the individual investigator. The "link" between observations of some aberration or group of symptoms and the selection of a good model system is often fortuitous or dependent upon personal contacts (Lewin, 1984). Whether this is the result of a total lack of published information (many negative results go unpublished) or a failure by Federal agencies to disseminate information available to them or a failure by the investigator to aggressively pursue obscure sources, the result is the same: time, money and animals are wasted. The peer review system helps to control waste, but it is not infallible and is frequently not even a participant when research funds are provided within clinical departments in medical centers. Hopefully, as the mastery of computer technology increases, data banks within the private and public sectors can cooperate to provide access to more complete information.

Standards for Research Personnel Education/Training

The standards of biomedical education in statistical insights and methodology and in basic animal technology are poor. Many investigators, graduate students, and technicians are uninformed in areas of medical information directly associated with their research; they may know little about the unsolved problem areas in human medicine and even less about the range of animal diseases and response to diseases. Veterinarians and human physicians are frequently uninformed of relevant medical information from each other's fields and lack training in techniques for data management and experimental design (Schwabe, 1978). Access to and acceptance of comparative information, comprehensive methodology training, and research ethics education are equally important to the practice of good biomedical research.

Any attempt to require even the most basic continuing education program in the deficient areas is sure to meet with stiff resistance. Institutions will never have the money and research personnel will lack the time; but until some remedial measures are taken, education will continue to be the most ignored path to more efficient, humane use of animals.

Conclusions

Many and varied modifications and alternatives to the use of animals in biomedical research are available: their numbers are increasing constantly, due primarily to the competition between investigators to be first with the best method for an advantage both in getting published and in securing continued grant support. While an emphasis on humane considerations carries more influence now than it has in the past, it is unlikely to be a major influence in the development of the vast majority of new methods.

Suggestions for greatly increased support specifically directed toward the development of alternatives are met with a good deal of skepticism within the biomedical research community. Even if funds were distributed judiciously, it is felt that projects of this nature would tend to attract the more inexperienced researchers. If so, both good and bad might result from such attraction. An inexperienced investigator might be more easily led into unproductive areas, be less well-grounded in good laboratory practices and procedures, and be less likely to recognize the value of serendipitous events; he is also more likely to try fresh, unbiased approaches to research and be more open to new ideas and to training in comparative medicine, methodology, and ethics.

The use of animals in biomedical research will, of necessity, continue into the foreseeable future: the major question to be resolved, therefore, is not if we will use them, but how they will be used and who will oversee their use.

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ALTERNATIVES TO THE USE OF ANIMALS IN BEHAVIORAL RESEARCH

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CHAPTER 1

GENERAL INTRODUCTION

Behavior

The definition of behavior used in this report has been taken in modified form from Alexander (1975). Behavior encompasses all of the movements and sensations by which organisms mediate their relationship with both living and nonliving components of the environment. The environment is taken to include not only objects and events external to the organism, but internal events as well (e.g., visceral cues, thoughts, emotions, etc.).

It is important to emphasize at the outset that behavior is not an object or a thing. Behavior is a process. It is a process which remains ongoing in most organisms until they die. Even sleep is a form of behavior. Unlike coloration, dentition, or size, behavior is a dynamic property of organisms which functions primarily to allow the organism to adapt to changing environmental conditions.

For the sake of organization, behavior can be subdivided into a number of different categories. Table 1 lists the categories of behavior which were used to prepare this report.

Legend

+	yes
+/-	possible
-	doubtful
0	no

Table 1

Alternatives to the Use of Animals in Behavioral Research

BEHAVIORAL CATERGORIES	Continued use of animals	ALTERNATIVES Continued but modified use of animals	Use of living components	Use of non-living systems	Use of computer programs
Abnormal behavior	+	-	0	0	0
Aggression	+	+/-	-	0	0
Animal movements	+	+/-	-	0	+/-
Body maintenance	+	+/-	-	0	0
Cognition	+	0	0	0	0
Communication	+	-	-	0	-
Exploration/ activity	+	+/-	-	0	0
Habitat and food selection	+	+/-	-	0	-
Learning, memory, problem solving	+	+/-, -	-	0	0
Motivation and emotion	+	-	0	0	0
Predator-prey relations	+	+/-	+/-	0	0
Reproduction, parental care	+	-	-	0	0
Sensation and perception	+	-	-	0	0
Social behavior	+	-	-	0	0
Spacing mechanisms	+	+/-	-	0	-

Definition of Behavioral Categories

Abnormal Behavior. In the broadest sense abnormal behavior is any behavior which deviates from normal patterns. Instances of abnormal behavior among animals might include unusually high levels of infanticide in mice, seemingly suicidal self-induced beachings by whales, phobic and neurotic problems in pets, or various laboratory-induced animal models of human psychopathology (e.g., catatonia, depression, drug addiction, obesity).

Aggression. Aggression can be defined as an organism's threatening to inflict, attempting to inflict, or actually doing physical harm to another organism.

Animal Movements. Animal movements represent major changes in location over time and space, such as patterns of migration, herding, homing, navigation, orientation, and dispersal.

Body Maintenance. Behaviors which function to provide body maintenance include hunger, thirst, respiration, thermoregulation, excretion, grooming, preening, and parasite removal.

Cognition. Although this category has been used indiscriminately to refer to practically all aspects of learned behavior (e.g., Mellgren, 1983), the use of the term here is restricted to instances of apparent mentalistic activity in animals (e.g., consciousness, thinking, imagery, self-awareness, intention, attribution).

Communication. Communication consists of an exchange of information between two or more organisms which results in a change in behavior. Instances of such information exchange can range from those which are stereotyped and instinctive (such as the dance "language" of honeybees) to those which might appear to have a symbolic basis (as in the case of the recent attempts to teach chimpanzees various forms of sign language). Depending on species, communication can involve visual, auditory, olfactory, and tactile cues.

Exploration/Activity. In addition to instances of curiosity and exploratory behavior, patterns of activity included in this category would consist of circadian rhythms, sleep, hibernation, roosttime restlessness, and different patterns of locomotion (e.g., swimming, brachiation, flying).

Learning, Memory, Problem Solving. These behaviors are represented by the acquisition and retention of new information which allows organisms to anticipate recurring environmental events, as well as changes in behavior which maximize or minimize certain outcomes. Included in this category would be the cultural transmission of information from one generation to the next and imitation.

Motivation and Emotion. The study of motivation is the study of mechanisms and manipulations which activate and sustain behavior. Emotion typically includes reactions which accompany different motivational states, and is often associated with such categories as fear, anxiety, apprehension, pleasure, and rage.

Predator-Prey Relations. As a consequence of selective pressure associated with predation, many prey species have developed an extensive and elaborate array of predator defenses couched in terms of sensory and/or behavioral adaptations (e.g., freezing, tonic immobility, burrowing). Likewise, predators employ a variety of behavioral strategies in prey identification and capture.

Reproduction, Parental Care. Patterns of courtship, mate selection, copulatory behavior, nest building, nurturing and care of offspring all fall within this category.

Sensation and Perception. How animals detect and interpret their environment is what is meant by sensation and perception. Topics included here would involve studies of sensory mechanisms, the development of search images, and highly specialized sensory mechanisms (e.g., echolocation).

Social Behavior. Social behavior is defined by a situation in which the behavior of one organism serves as a stimulus for the behavior of another, and vice versa. Instances of social behavior could range from simple forms of aggregation to complex exchanges among individuals (e.g., dominance, cooperation, reciprocal altruism),

Spacing Mechanisms. Spacing mechanisms are intimately tied to social behavior, and range from such topics as individual distance to the establishment and maintenance of territories.

Methods of Studying Behavior

Instances of behavior in any of the above categories can be studied from a number of different perspectives.

Field Studies. Field studies represent an attempt to examine the behavior in question as it occurs under natural circumstances. Such studies do not typically involve attempts to manipulate or control the conditions of observation. Field studies provide an important descriptive base, and can be a valuable source of hypotheses about behavior which can be subsequently tested under laboratory conditions.

Developmental Studies. Among many species the emergence of different patterns of behavior is a reflection of both maturational and experiential factors. Developmental variables have been identified as being important in the expression of such diverse behaviors as aggression, communication, activity, learning, and many aspects of social behavior.

Laboratory Studies. Laboratory studies undertake to manipulate and control the conditions of observation so as to more precisely specify the variables and conditions which influence the behavior in question. Most laboratory studies of behavior can be subdivided into two categories: (i) those that attempt to identify the environmental determinants of behavior, and (ii) those concerned with the organic basis for behavior. Within the latter approach one can identify a number of approaches involving attempts to identify the neuroanatomical, neurochemical, endocrinological, and genetic underpinnings for behavior.

Naturalistic Observation

Observation of animals living under natural conditions has frequently been suggested as an alternative to the use of animals in laboratory research (Bowd, 1980; Pratt, 1980). The following benefits and limitations of naturalistic observation are now widely recognized (Miller, 1977).

1. Natural observation is frequently a starting point. Observations of animals in the field provide an important descriptive base and can be a valuable source of hypotheses which can be tested under laboratory conditions.

2. Naturalistic observations can be used to compare behavior observed in the field with that occurring in the laboratory to assess the extent to which an artificial environment may alter behavior and whether the results will be generalizable.

3. Field studies can increase the efficiency of utilization of animals by providing useful information on species variables and biological constraints on behavior.

4. The principal drawback to naturalistic observation is the absence of control. Under natural conditions events frequently change in both important and spurious ways, and in the face of such covariation it is impossible to establish cause and effect relations.

5. Rather than an alternative to laboratory research, naturalistic observations are an important part of the whole process that culminates in the need to conduct experiments.

Behavioral versus Biomedical Research

While it is commonplace, the distinction between behavioral and biomedical research frequently breaks down. Behavior, in the last analysis, is a biological phenomenon. Behavior presupposes a living organism, and how it behaves is influenced in complex ways by its genetic makeup, hormonal status, physiology, and neurochemistry. Intervening between environmental events as input, and behavioral events as output, are complex neuroanatomical networks involving receptor surfaces, electrochemical reactions, autonomic influences, and effector organs. Behavior does not occur in a vacuum. Biology is what makes behavioral events possible.

As support for this view, it is increasingly apparent that many aspects of behavioral research have important biomedical implications. While an exhaustive account would be inappropriate in this context, suffice it to say that problems associated with obesity, hypertension, drug addiction, headaches, aggression, enuresis, alcoholism, sexual dysfunction, brain damage, epilepsy, schizophrenia, separation induced depression, learning disorders, myopia, smoking, anorexia nervosa, suicide, stomach ulcers, sexual assault, mental retardation, as well as a variety of other psychological disorders are now much better understood as a consequence of behavioral research on animals (e.g., Maser & Seligman, 1977; Keehn, 1979, 1982; Elkins, 1980; Sullivan & Willard, 1978; McKinney & Moran, 1981; Katz, 1981; Kaufman & Rosenblum, 1969; Breese, Mueller, Mailman, Frye & Vogel, 1978; Gaito, 1979).

Use of Animals in Behavioral Research

There are a number of important reasons for the use of animals in behavioral research.

Control. Under laboratory conditions it is possible to manipulate and control a variety of factors which in different settings would confuse, contaminate, and confound any attempt to interpret a behavioral outcome. The effect of exposure to alcohol in utero is a case in point. Without the use of animals it would be difficult if not impossible to identify in a conclusive way the deleterious effects of alcohol on the developing fetus. Among humans the consumption of alcohol is correlated with a variety of other factors which could produce teratological effects in their own right. For instance, people who consume alcohol are also more likely to smoke tobacco, drink coffee, consume other drugs, show dietary differences, and in general may have lifestyles which differ in many ways from persons who do not (Riley & Meyer, 1984). The advantage of using animals to examine fetal alcohol effects is that all of these as well as other factors can be controlled in such a way that one can examine the effects of alcohol alone on fetal development. Indeed, it has now been established that fetal exposure to alcohol in amounts considerably less than what is required to produce physical damage, can still lead to substantial behavior effects which show up later in life. Behavioral testing for drug effects on animals in utero promises to provide a much more sensitive means of screening compounds for possible teratological and toxicological effects (e.g., Riley & Meyer, 1984).

With animals it is also possible to control for such things as genetic background, prior experience, temperature, humidity, diet, previous social encounters, and a host of other factors, which might otherwise render the effects ostensibly produced by the experimental manipulation uninterpretable.

Objectivity. A basic requirement of any science is that it be objective and impartial. Especially in behavioral research this is of paramount importance. When the subject matter and the scientist are members of the same species (i.e., humans studying humans) not only is it more difficult to remain objective, but there are a variety of other complications which arise from the social relationship which exists between those conducting the research and those participating as subjects (e.g., Rosenthal, 1977).

Developmental Effects. Among many species behavior changes as a function of age. The problem this poses for human research is one of time. Human development continues throughout the lifespan. To chart such changes within the same individuals would take decades of research involving exhaustive follow-up studies and the everpresent danger of losing subjects (because of death, relocation, etc.). The alternative to such longitudinal work is to conduct cross-sectional studies, where current samples are drawn from different age groups. The problem with this is that there are invariably sociological and cultural changes over time which confound any apparent difference between age groups (e.g., fifty years ago an eighth grade education was the norm). Because of greater control and protracted life spans among some laboratory animals (e.g., rats and mice) such problems can be circumvented altogether or at least minimized.

Genetic Effects. There is growing evidence for a variety of genetic effects on behavior (e.g., Hirsch, 1967). With animals it is possible to do careful selective breeding studies to establish, pinpoint, and quantify such effects.

Methodology. The fact that animals cannot talk might seem at first glance like a serious disadvantage to conducting behavioral research with animals. But in a very real sense the study of animals helps to keep us honest about the study of our own behavior. The use of animals forces the behavioral scientist to develop objective, operational definitions coupled with rigorous research techniques which can later be applied to humans.

Complexity. Behavior, especially human behavior, can be extremely complex. The use of animals which are less complex represents a means of being able to identify some of the basic elements and principles of behavior which might otherwise remain inextricably embedded in a mosaic of other factors.

Ideal Case. Certain behavioral phenomena simply fall outside the realm of our own sensory or motor equipment. Echolocation, infrared detection, homing, and flight are all examples of behavior which require the use of certain animals as subjects for research purposes.

Heuristic Value. Research on the behavior of animals has been an important source of hypotheses about human behavior and an impetus to research on humans (e.g., Maser & Seligman, 1977). Much of what is now known about the principles of learning was initially derived from research on animals. Likewise, the use of a variety of therapeutic techniques (e.g., desensitization) was derived from work with animals.

Practical Applications. As detailed in a subsequent section, research with animals has also led to findings which have important implications for animals themselves (e.g., farm animal production, animal husbandry, treatment of animal behavior disorders, use of animals to solve ecological problems).

Ethical Considerations. There are obviously certain things which simply cannot be done to humans. Problems associated with such diverse issues as child abuse, mental illness, drug addiction, brain damage, and sexual assault simply cannot be dealt with in an experimental setting with humans (in the sense of direct manipulation of the phenomenon in question). Yet these are problems with vast social, psychological, medical, and economic consequences which involve untold amounts of human suffering. As detailed elsewhere in this report, the use of animals in behavioral research has begun to shed important light on many of these issues.

General versus Restricted Behavioral Phenomena

Many behavioral phenomena appear to cut across different species. Patterns of migration, for example, are common to such diverse groups as insects, fish, birds, and even some species of mammals. Much the same appears true for learning, motivation, and bodily maintenance. However, that is not to say that in all instances the end result is achieved by the same means. Indeed, the more we learn about different behavioral phenomena the more apparent it becomes that the differences between species are considerable. Generalizations about categories of behavior in unrelated species (e.g., parental care, hoarding) are misleading because they evolved independently (see Lockard, 1971). Moreover, comparing the performance of different species on a simple task often has no bearing on larger issues such as intelligence (Kalat, 1983). Finally, what used to be seen as general principles and "laws" of learning now turn out to be specific to certain species under certain situations (Bitterman, 1965, 1975; Seligman, 1970).

Then there are behaviors which are even of more limited scope phyletically but of profound importance in terms of their bearing on the question of human behavior. For instance, the capacity to recognize one's own reflection in a mirror has only been found in humans, chimpanzees, and orangutans and much the same may apply to instances of intentional deception, gratitude, grudging, sympathy, empathy, attribution, reconciliation and sorrow (see Gallup, 1983).

Thus, in the latter case there simply are no alternatives, while in the former case the alternatives may turn out to be highly misleading and fail to represent the phenomena in question.

Rationale for the Use of Animal Models

Animals are often used to model various human behavioral, psychological, and psychosomatic disorders for a number of reasons. Among humans, unlike animals, confounding factors can rarely, if ever, be controlled (e.g., past experience, diet, genetic makeup). Second, epidemiological studies are extremely expensive and very time consuming (Riley & Meyer, 1984). For example, certain chemicals ingested by pregnant females may produce psychological problems (e.g., learning disabilities) in offspring later in life, but among humans it would take decades to even detect such effects. Also, because of moral and ethical considerations, many studies cannot be conducted on people; e.g., in developing a theory of schizophrenia one could not test that theory by trying to induce schizophrenia in humans. Finally, it is the case that many abnormalities occur at low frequencies. Thus, if prenatal exposure to alcohol caused the incidence of a behavioral anomaly to double, which had a spontaneous rate of occurrence of 0.1% in the population at large, the sample sizes which would be needed to establish such an increase would have to be extremely large (Riley & Meyer, 1984).

It is also important to acknowledge that an animal model may never be exactly the same as the condition being modelled. A model is an experimental compromise created to help understand complex phenomena (McKinney & Moran, 1981). Animal models of behavioral disorders can be evaluated using the following criteria (see McKinney & Moran): 1) the similarity of inducing conditions, 2) the similarity of behavioral states produced, 3) the presence of common underlying neurobiological mechanisms, and 4) the extent to which the behaviors are similarly affected by clinical treatments.

To counter objections that animal models of human behavioral disorders are invalid because the animals do not normally perform such behaviors under natural conditions, Siegel (1979) has shown in hundreds of cases that animals ingest drugs (which are typically plant produced) in their natural habitat. Many of such drugs have marked psychoactive effects and are repeatedly ingested by animals, suggesting that such behavior is both intentional and addicting.

Advantages to Animals

In addition to their use in modeling a variety of biomedical and psychological problems in humans, research on animal behavior has in many instances led to techniques and findings which benefit the animals themselves. For instance, an understanding of behavior is crucial for designing optimal captive environments for the protection and breeding of endangered species (e.g., Snowdon, 1983). Increased attention has also been paid to the behavior of farm animals (Curtis & McGlone, 1982; Kilgour, 1976). The study of mother-infant attachments, social behavior in groups, stress resulting from overcrowding and confinement, and habitat preferences has led to important insights into farm animal welfare and husbandry (e.g., Simonsen, 1982).

It is also important to note in this context that much of what has been learned about behavioral problems in humans through animal research is now also being applied to the animals themselves. The treatments of aggressive problems in cats (Borchelt & Voith, 1982), as well as fears and phobias in dogs (Hothersall & Tuber, 1979; Tuber, Hothersall & Peters, 1982) have been shown to be highly effective using these techniques.

Finally, a knowledge of animal behavior has been shown to be of fundamental importance in attempting to identify and solve ecological problems which could ultimately impinge on the survival of all species, including ourselves. The discovery and subsequent synthesis of sex pheromones has begun to revolutionize our ability to control pests which otherwise devastate crops. Moreover, rather than having to use toxic pesticides applied over vast areas, it is now possible to bait poisoned traps with specific pheromones so as to preclude environmental contamination.

In another ingenious application of laboratory findings to the solution of ecological problems, techniques based on taste-aversion learning in rats (e.g., Garcia & Koelling, 1966) deserve extended comment. By pairing chemical or radiation induced illness with different flavors, it was discovered that rats could develop highly durable taste aversions in only one or two trials, and that up to an hour or more could elapse between the ingestion of a novel food and the onset of illness without disrupting such learning. By pairing lithium chloride induced illness with flesh of different prey species it is now possible to control predation by coyotes on sheep and turkeys (e.g., Ellins & Catalano, 1980). Indeed, one or two trials is sufficient to eliminate attacks on specific domestic farm animals, but leaves the coyote free to attack alternative prey (Gustavson, Garcia, Hankins & Rusiniak, 1974). This has been recently extended to reducing crop damage by crows, and even appears to have promise for dealing with cancer patients undergoing radiation therapy (see Adams, 1984). In the latter case, a frequent complication has been that the radiation produces gastro-intestinal illness which generalizes to all food and renders such patients unable to eat. It is now possible to circumvent this problem by restricting patients to one particular kind of food during radiation treatment, so that the aversion which develops is specific to that food alone. This approach has also been used with considerable success in treating chronic alcoholics (Elkins, 1980). Thus, behavioral research with rats under laboratory conditions has led to positive economic consequences in terms of predator and pest control, which benefits the animals themselves, precludes the need to kill predators, and can even be applied to people with highly beneficial biomedical consequences.

Other Benefits

It is also important to realize that the use of animals as a means of helping handicapped persons deal with their disabilities has often relied on a knowledge of animal behavior. Familiar examples include the use of guide dogs for the blind (Goddard & Beilharz, 1982), and training capuchin monkeys to perform as aids for quadriplegics (Willard, Dana, Stark, Owen, Zazula & Corcoran, 1982). Pets have also been shown to have therapeutic value for psychiatric patients (Corson, Corson, Gwynne & Arnold, 1977), and may even have promise for alleviating the depression resulting from child loss (Suarez & Gallup, in press).

Why are Particular Species Used in Behavioral Research?

While the number and variety of different species used in behavioral research is very large, some species have been used more often (e.g., rats) than others. The reasons for this are worth detailing.

1. Some species are much more available than others. For instance, in the case of rats and mice there are large numbers of commercial breeding businesses which can supply animals of particular strains, ages, and sex.

2. In many instances existing data bases have been built upon a particular species, thus additional work, in order to contribute to the field in a direct way, needs to employ the same species.

3. Clearly, non-endangered commercially available animals are preferred for most purposes over endangered ones.

4. Some species may exhibit the behavior of interest in a more vivid and robust form than others (e.g., echolocation in bats).

5. There are certain behavioral phenomena which are only shown by a limited number of species, such as self-recognition or tool fabrication (see Gallup, 1983).

6. There is also the question of cost and maintenance. Chimpanzees are very expensive and difficult to maintain, whereas rats are readily available and relatively easy to house.

7. Finally, there is the issue of generalizability. Because we are more closely related, behavioral research with primates is

often thought to be more applicable to humans, than comparable work with frogs, worms, or fish.

It should be obvious that any attempt to identify alternatives or substitute one species for another must take each of these considerations into account.

Surveys of Animals Used in Behavioral Research

Although some writers have created the impression that animals are being used with increasing frequency and that they represent the predominant subjects in most behavior research (e.g., Bowd, 1980), the data show otherwise. A survey of articles appearing in the 1939 and 1979 volumes of the Psychological Abstracts revealed that only 9.97% of the 1939 articles and 7.4% of the 1979 articles used animals as subjects (Gallup & Suarez, 1980a). Thus, the relative frequency of use of animals in behavioral research has not increased dramatically in the latter half of this century (if anything there has been a modest decline), and fewer than ten percent of the journal articles in psychology deal with animals.

As to the kinds of animals used in behavioral research, Beach (1950) examined studies appearing in the Journal of Animal Behavior from 1911 to 1917 and its successor, the Journal of Comparative Psychology from 1919 to 1948. Fifty percent of the articles dealt with rats, 31% involved mammals other than rats, 10% used vertebrates other than mammals, and 9% reported results obtained from invertebrates. Numerous follow-up surveys have been conducted since Beach published his original findings (e.g., Schneirla, 1952; Dukes, 1960; Schrier, 1969; Grossett & Poling, 1982), and they all agree in showing a stable preference for the use of rats as the principal subjects in behavioral research with animals.

Indeed, the more recent surveys generally show that about 60% of the articles published by psychologists on animal behavior involve rats.

It is important to distinguish between the number of published articles involving different species and the actual number of animals used in research. Some papers might be based on only a few subjects, while others might employ hundreds of animals. Moreover, depending on species and type of research, some animals might be employed in multiple experiments (e.g., in primate research it is not uncommon for animals to be used in a succession of either related or unrelated studies over periods of years, while this would rarely be the case for rodents). Mesirow (1984) recently conducted a survey of graduate departments of psychology in this country in an attempt to determine the number of animals held on hand for research purposes. The results of her survey are depicted in Table 2. As can be seen from this table, mice, rats, and birds comprise over 95% of all the animals used by psychologists. Dogs, cats, and primates combined, account for only 1.14% of the total.

Thus, one can conclude with some confidence that most psychological research on animals is already being conducted on "lower" mammals, and a substantial number of additional studies involve nonmammalian forms.

Table 2

Types and Numbers of Animals Housed in Graduate Departments of Psychology in the United States (adapted from Mesirow, 1984)

	Number	%
Primates	1,340	0.53
Rats/Mice	226,762	90.00
Dogs	51	0.02
Birds	14,415	5.70
Cats	1,502	0.59
Amphibians	841	0.33
Rabbits	967	0.38
Other (Hamsters, moles, gerbils, fish)	6,809	2.70
TOTAL	252,687	100.00

Increased Reliance on Animals

In some instances we may be progressing toward an increased reliance on the use of animals in behavioral research. The growing field of behavioral teratology is a case in point. Because of the thalidomide tragedy, there is growing public concern about the effects of drugs and other chemicals on the unborn fetus. It has now been determined that ingestion of alcohol by pregnant rats, in amounts appreciably less than what is required to produce detectable physical abnormalities on the part of offspring, can nevertheless produce reliable behavioral deficiencies which show up in later life (e.g., Riley & Meyer, 1984). Thus, behavioral testing has recently emerged as an even more sensitive means of detecting the possible teratological effects of a variety of compounds. According to Vorhees (1983) both Japan and England have enacted government regulations which now mandate behavioral testing for teratogenic effects on all new drugs prior to marketing. While such requirements have yet to be imposed in this country, the evidence suggests that U.S. regulatory agencies are giving this serious consideration. Vorhees (1983) summarizes this evidence as follows: "1) Consideration of behavioral teratogenic evaluations by the 'Reproduction and teratology guidelines: Review of deliberations by the National Toxicology Advisory Committee's Reproduction Panel' (Collins, 1978), 2) FDA sponsorship of research to develop a developmental psychotoxicity/behavioral teratogenicity screening test battery, and 3) FDA sponsorship of an interlaboratory behavioral teratology research protocol. There is also evidence that the U.S. EPA is giving behavioral teratology assessment serious consideration. This evidence includes: 1) EPA sponsorship of a workshop on 'Test methods for definition of effect of toxic substances on behavior and neuromotor function (Geller et al., 1979), which included considerations for behavioral teratogenesis testing; and review by the EPA of the need for behavioral teratogenic testing on chloromethane and other chlorinated benzenes." (p. 469).

If such developments materialize, it will clearly lead to an increased use of animals in behavioral research.

Meaning of alternatives

It is important to be clear at the outset about the various topics which are dealt with in this report. Alternatives to the use of animals in behavioral research implies the use of anything other than animals (e.g., plants, computer simulations, tissue cultures, nonliving systems). The use of a "lower" species to replace a "higher" one is not strictly speaking an alternative to the use of animals in behavioral research, but rather an alternative to the use of one animal as opposed to another. Similarly, to substitute one pain producing stimulus, such as electric shock, for another (e.g., formalin injections), is not an alternative to the use of animals in behavioral research. This involves the use of an alternative method which is equally effective, but might conceivably be more humane. Finally, it is also important to note that any procedure which is designed to reduce the number of animals used in research is again not an alternative to the use of animals per se, but simply a means of achieving the same result through the use of fewer subjects. Thus, although this report is entitled "Alternatives to the Use of Animals in Behavioral Research", it encompasses considerations which cannot be subsumed by the title.

The importance of the foregoing distinctions is that under the category "continued, but modified use of animals", should a procedure or technique surface which appears more or at least as viable as one currently in use, or should it be the case that one species will provide data which are just as sound as those typically collected from another, we would not want these revelations to be misconstrued as alternatives to the use of animals in behavioral research. Rather, they should be taken only for what they in fact represent.

CHAPTER 2

CONTINUED BUT MODIFIED USE OF ANIMALS

Reduction in the Number of Animals Used

Statistical Considerations. Individual members of every species vary in their behavior both between each other (between subjects) and, in the case of a single individual, over time (within subjects). The goal of an experiment is to identify patterns that remain when these sources of variability have been eliminated or controlled. Ideally, one would like to be able to conclude that the observed effects are due to the conditions being manipulated in the experiment and not to extraneous factors. This decision usually rests on the outcome of statistical tests.

The power of a statistical test is an index of its sensitivity to detecting experimental effects. The most widely recognized method of increasing power and, hence, the sensitivity of an experiment is to use a large sample of subjects. Typically, the more variable the results, the less power, and therefore the need for large sample sizes. While it is impossible to determine the magnitude of variability prior to conducting the experiment, one can sometimes estimate the amount of variability likely to be encountered from pilot studies or previously published research in the same or related areas. Once an estimate of variability has been obtained, there are statistical tables available which one can use to determine the sample size needed to attain certain levels of power (Winer, 1971).

In certain instances, however, there are other methods of increasing power and reducing the number of animals needed. A discussion of these methods along with their advantages and disadvantages follows:

1. Theoretical methods of increasing power.

a. Choosing a lower level of statistical significance (i.e., the likelihood that the results were due to chance) increases power and reduces the number of subjects needed. However, this also increases the chances of concluding that the experimental procedure produced an effect, when in fact it did not. Thus, the experiment would have to be repeated a number of times to establish the reliability of the results, and any initial gain in terms of fewer subjects might be lost.

b. The use of greater precision in the conduct of an experiment may reduce variability and increase power. For instance, highly precise behavioral measurements coupled with the elimination or control of extraneous variables would reduce the need for large numbers of subjects (e.g., Still, 1982).

c. The sensitivity and power of an experiment is increased if the data are analyzed by parametric rather than nonparametric statistical tests (Still, 1982).

d. Better experimental designs can increase power. Factorial designs, for example, where two or more treatments are manipulated concurrently, are more powerful and should be used instead of testing the effects of different treatments in separate experiments. Not only does the use of factorial designs increase power, but they require fewer control subjects. However, it is important to note that in areas that have not been heavily researched there are inherent dangers to the use of factorial designs. In the absence of sufficient background information on the effects of the treatment variables administered individually, there may be no effect of the treatments when given in combination (i.e., one may cancel out the effects of the other), the result being that in attempting to use fewer animals, all are used needlessly.

e. Power is increased as the magnitude of the treatment effect is increased. Treatment effects can be maximized by choosing widely spaced levels of the treatment variable or by including conditions that are thought to maximize the appearance of the phenomenon under study (Keppel, 1973).

2. Reducing variability by using within-subjects designs. Many experiments on animal behavior are conducted using between-subjects designs. That is, different groups of animals are given different treatments and the performance of the different groups are compared. However, individuals also vary in their behavior. Depending upon the degree of variability, large numbers of subjects

may be needed in each group to obtain statistically significant results.

Alternatively, under certain conditions one can employ a within-subjects (or repeated measures) design, which uses only one group of animals instead of multiple groups. Under these conditions all members of the group serve in all treatment conditions. The advantage of this technique is that it minimizes variability by controlling for individual differences. However, there is a major drawback to the use of within-subjects designs, namely the possibility of contaminating the data and nullifying the results as a consequence of carry-over effects. That is, treatments already received by a subject may influence and thereby confound performance under subsequent treatments. Carryover effects can be partially offset by counterbalancing, wherein the experimenter ensures that there is an equal occurrence of each experimental treatment at each stage of the experiment. This technique balances any effect of prior testing over all treatment conditions equally (see Keppel, 1973).

Although within-subjects designs are effective in reducing both variability and the number of subjects needed, the inherent danger of carry-over effects cannot in many instances be completely eliminated and, thus, this is not an appropriate design for many investigations.

3. Reducing variability by randomized block designs. The

basic technique in randomized block designs consists of assigning subjects to groups based on evidence of being similar to one another on one or more characteristics that are known to be related to the behavior under investigation. Two or more such blocks are formed and subjects from each block are assigned randomly to the treatment conditions. This design achieves a reduction in variability by restricting the degree of individual differences within blocks, and thereby increases power (Keppel, 1973).

Although randomized block designs are effective in reducing the number of animals needed in an experiment, they are not applicable to all areas of behavioral research. This technique is predicated on substantial prior knowledge of the behavior being investigated and therefore is limited to those areas which have been intensively researched.

4. Analysis of covariance. An analysis of covariance utilizes the same information as randomized block designs; i.e., it relies on scores from a correlated control variable. For randomized block designs these scores must be known before the experiment is conducted, whereas this is not the case for an analysis of covariance. The covariance procedure is applied to the data after they are collected to adjust for chance differences among groups on the control variable. By reducing variability which is associated with individual differences on the control variable one achieves more power, and fewer animals may be needed to obtain

statistically significant results (see Keppel, 1973 for more detail).

Single Subject Designs. In some instances it is possible to make inferences about populations from very small samples. This is a common practice in psychophysical experiments where there is a substantial prior body of evidence from which it follows that the behaviors under investigation do not vary appreciably within the population at large (e.g., visual sensitivity to light). Although such experiments can be conducted using a single subject, two or three are typically used to guard against the possibility of misleading results from an atypical subject (see Still, 1982).

In other than psychophysical experiments, the general procedure in single-subject research consists of 1) choosing a baseline, which involves measuring the frequency of occurrence of the target behavior in question, 2) changing one treatment variable at a time, 3) temporarily withdrawing the experimental treatment to assess its causal effects, and 4) repeated measurement of the baseline behavior before and after each treatment. There are even more sophisticated experimental designs available for single-subject research (see Hersen & Barlow, 1976). Statistical analyses are reported infrequently, however, due to the absence of many statistical techniques for handling such data. But some acceptable methods are available, such as using time series analyses to test for changes over time (Kazdin, 1976). Single-subject designs have come to be used with increasing frequency in many areas of animal operant conditioning and human clinical research (e.g., Sidman, 1960).

The obvious limitation to studying a single subject is the generality of the findings. This is commonly dealt with by replicating the experiment a number of times with different subjects (Hersen & Barlow, 1976). Thus, although single-subject designs would appear to be a means of reducing the number of animals used in behavioral research, the need for replication often negates or at least compromises this apparent advantage.

Use of Inbred Strains. One way of reducing variability (and hence increasing power) is to use highly homogeneous populations of subjects. Inbred strains of rats and mice, produced as a result of 20 or more generations of brother-sister matings, represent one means of achieving this objective. In most inbred strains all individuals are genetically identical (isogenic) and genetically stable; i.e., they only change as a result of the slow accumulation of mutations (Festing, 1981). In contrast, outbred stocks of animals are genetically variable. They contain an unknown and uncontrollable degree of genetic variation which may obscure or mask experimental treatment effects. Inbred strains not only have the virtue of increasing statistical power, they also reduce variability between experiments conducted in different laboratories or in the same laboratory at different times (Festing, 1981; Lockard, 1968).

Alternatively, it can be argued that experiments should employ animals drawn from heterogeneous outbred populations in order to get a broad genetic basis for results that can be extrapolated (e.g., to man). However, differences between inbred strains are usually greater than the differences between individuals of an outbred stock. Greater generality may, then, be obtained by conducting experiments with two or more inbred strains (Festing, 1981). It should be noted, however, that inbred strains are usually much more expensive than outbred animals.

Considerable controversy exists over the advantages and disadvantages of using genetically undefined or random source pet species, such as dogs, obtained from animal shelters in research (see Rowan, 1984). One perspective acknowledges that while genetically defined animals may produce more precise experimental results, undefined "pound" dogs may be a better utilization of resources since humane societies in the U.S. are killing millions of dogs per year. Thus, using animals for research that would otherwise be destroyed would reduce the waste of life (Rowsell, 1982).

Sharing Animals. A team approach to research questions across scientific disciplines might reduce the number of animals needed for research (e.g., Rowsell, 1975). For example, researchers studying a behavioral phenomenon by noninvasive means could share their animals at the conclusion of the experiment with biologists investigating the anatomy or physiology of that species. Likewise, scientists from different disciplines could collaborate on the formation of research proposals. For instance, a psychologist may be interested in studying predator-prey relations, while a biologist wants to study endocrinological changes in response to stress. Effective collaboration between such individuals could result in two different data sets obtained simultaneously from the same animals.

Importance of Replication and Information Retrieval. The ultimate test of the reliability of any finding is replication. Because of this the author of any scientific paper is obliged to provide enough information about the way the data were collected to provide others with the opportunity to replicate their findings. However, the brevity of papers dictated by some journals makes it difficult to spell out every detail, sometimes leading to an inability to replicate another investigator's findings (Meier, 1968). As a consequence, such omissions can lead to a waste of animals.

In other instances scientists have been responsible for using more animals than necessary because of a lack of familiarity with the existing literature. For example, a researcher studying the effects of a certain drug on a particular behavior may have been unaware of previously published studies using that drug. Had he first consulted the relevant literature he would have been able to determine optimal dose parameters, rather than conducting a series of pilot studies on any number of animals.

Computerized bibliographic searches and the more efficient use of easy access information retrieval has the potential to reduce the number of animals used in research since it would eliminate needless duplication. Further developments and improvements in information retrieval could have similar effects.

Pain

Ultimately, any attempt to understand pain, let alone alleviate pain, will require research on pain. But even among members of our own species pain is an elusive phenomenon. It is difficult to assess, objectify, and measure. Problems posed by congenital insensitivity to pain, phantom limb pain, and malingering further confuse things, not to mention the existence of substantial individual and cultural differences in pain sensitivity.

It is a fairly simple matter to describe and measure reactions to noxious stimuli, but pain is a subjective phenomenon. There is simply no way for one person to experience another person's experience, let alone the experience of species other than our own. Experience is an intrinsically private event. The only experience you can ever experience is your own experience. To illustrate the kinds of problems this poses for research, consider the traditional methods of measuring pain sensitivity in animals. Most studies of pain in animals employ what are called flinch and jump thresholds, which are used as a means of indexing the minimal amount of electric shock or heat needed to produce a reaction. The rationale underlying this approach relates back to the fact that pain is always an inference. The problem, however, is that under such circumstances sensitivity to pain is confounded with reactivity to pain (Wallnau & Gallup, 1977). If, for example, an experimental drug changes an animal's jump threshold to electric shock, does that mean the animal is more (or less) sensitive to shock, or conversely more (or less) reactive to shock?

Thus, the study of pain in animals is always inferential. But for that matter, the same is true for humans. The difference is that in the latter case inferences are typically made on the basis of verbal reports.

Pain in an Ecological Context

The experience of pain is a highly adaptive capacity. Pain evolved to prevent organisms from engaging in behaviors which would otherwise prove maladaptive. Humans who are born congenitally insensitive to pain become terribly scarred and mutilated, often develop a sense of being invincible, and have short life expectancies (McMurray, 1955; Melzack, 1973; Sternbach, 1968).

Perhaps because pain plays such an essential role in regulating the behavior of organisms, pain thresholds are surprisingly consistent across a great diversity of species (Kitchell & Erickson, 1983). The discovery of endogenous opiates in earthworms (Alumets et al., 1979) and recent findings with spiders (Eisner & Camazine, 1983) suggests that even many invertebrates can feel pain. Thus, there is little or no evidence to date that the use of "lower" species will reduce the problems associated with pain and suffering.

As Dawkins (1980) points out, a commonly proposed index of suffering (as opposed to pain per se) is to assess the extent to which an animal is unable to perform behavioral patterns typical of its species. However, domestication of both laboratory and farm animals has produced major changes. What an animal is accustomed to can have a profound effect on what the animal regards as most desirable. For instance, hens living in battery cages prefer these even to an outside run with grass when first offered a free choice between the two. Moreover, as Dawkins shows, when animals can express a choice between captivity and the wild, they do not always prefer the latter and frequently return to their cages if allowed.

Another point that Dawkins (1980) makes is that using wild animals as a standard of welfare is a logical mistake, since it assumes that they do not suffer. Wild animals are continually exposed to disease, parasites, predation, starvation, and freezing. Moreover, death by predation may be extremely painful since some predators kill their prey by eating them alive. As a consequence, in many instances captive animals are much healthier than their wild counterparts.

Consider what life must be like for a rat living under natural conditions. Survival in a garbage dump is no mean task. It entails having to cope with the ever-present danger of being killed or maimed by predators, not knowing where your next meal will come from, dying of thirst should there be a drought, freezing to death in the winter, having to constantly compete and fight with other rats, being infested by mites and ticks, contracting any number of dread diseases, ingesting lethal poisons, suffocating should your burrow collapse, having your neck broken in a rat trap, or being run over by dump trucks and bulldozers, to mention only a few. Indeed, it may be the case, among species which have adapted to life under conditions of high stress, that common research procedures (e.g., electric shock, food deprivation, social isolation) contribute to their well-being (see Rowan, 1984).

It is also important to realize that what an animal perceives as painful or stressful may not always correspond to human judgments about such events. For instance, recent data (e.g., Suarez & Gallup, 1982, 1983) show that mere exposure to a human can produce effects which are equivalent to those ordinarily triggered by electric shock or loud noise (e.g., freezing, defecation, vocalization).

Although it is common practice to consider a stimulus as painful if it causes an animal to make an escape reaction (Vierck, 1976), there are instances in which animals will perform behaviors in order to receive electric shock as an apparent "reward" (Kelleher & Morse, 1968; McKearney, 1969). Moreover, such effects are only obtained if the intensity of shock is painful as judged by reactions to the shock in other contexts! Such experiments raise serious questions about the notion that pain is a universal punisher.

Types of Pain Producing Stimuli

Pain can be induced by means of mechanical, thermal, electrical, or chemical stimuli (Lineberry, 1981). Of the various types of pain stimuli used for research purposes, electric shock is the only one which does not produce tissue damage. Indeed, there are a number of reasons why electric shock ought to be the stimulus of choice as a pain producing event. Shock is easily quantifiable. The parameters of shock can be manipulated and specified with a high degree of precision over a wide range of parameters. Electric shock can be administered in such a way as to have a discrete or gradual onset and offset. Brief electric shock of the type most behavioral scientists use (less than .001 amperes) produces no physical damage, no serious injury, no disfigurement, no bleeding, no crippling, no tissue destruction, and no long-term painful aftereffects.

Such advantages, however, need to be tempered by the realization that electric shock is a highly atypical stimulus (Gallup & Suarez, 1980b). No contemporary terrestrial species appears to have evolved under conditions of electric shock. Thus, whether data obtained using electric shock will generalize to other situations involving pain is an empirical question that remains unanswered.

Reduction in the Degree of Pain or Insult

While there have been numerous charges of widespread abuse of animals in behavioral research (e.g., Bowd, 1980; Mobilization for Animals, 1984), recent surveys paint a much different picture. For example, Coile and Miller (1984) did a survey of all articles appearing during the last five years in journals of the American Psychological Association which typically publish animal research (e.g., *Journal of Comparative and Physiological Psychology*). Out of a total of over 600 articles reporting psychological research on animals, only 3.9% of the studies used electric shock of intensities greater than .001 of an ampere. Moreover, of the studies which employed electric shock, most used rodents; only one-half of one percent involved monkeys, dogs, or cats.

In the case of deprivation procedures, none of the articles surveyed used periods of food deprivation which lasted longer than 48 hours, and less than one percent were in excess of 24 hours. None of the studies maintained animals below 70% of their normal body weights, and a mere 3.6% entailed reductions below 80% body weight. Of the studies in the latter category only 0.3% used mammals.

Far fewer studies used water deprivation. Only 0.7% deprived animals of water for more than 24 hours, and none for longer than two days. In not a single instance were animals starved to death, nor did any of the animals die of thirst.

The survey by Coile and Miller revealed no instances in which animals were smashed or crushed, nor did any of the animals have their limbs mutilated or amputated. None were maintained in isolation for purposes of being driven insane.

The following represents some of the recommendations which have been made to reduce pain and/or discomfort in animal experiments involving aversive stimulation.

1. The lowest possible level of electric shock should be used that will at the same time maintain the behavior under study (Davis, 1981). It should be noted, however, that this may reduce power and require a large sample size (see section on statistics).

2. To the extent possible animals should be given predictable rather than unpredictable shock, and should be given an opportunity to control the termination of shock (Davis, 1981). Rats, for example, will choose to receive more shocks at greater intensity in order to receive a warning cue prior to each shock delivery (Badia, Culbertson & Harsh, 1973).

3. If aversive stimulation must be used, consider alternatives to electric shock such as loud noise, bright lights, or species-specific threat stimuli (Lea, 1979).

4. In developing models of chronic pain, the model should closely simulate a particular chronic pain syndrome in humans (e.g., arthritis, cancer). Otherwise there is no justification for the procedure (Kerr, 1982).

5. Ideally the animal should have an opportunity to control the intensity of the stimulus in chronic pain studies. While the objection to this might be that, given this option, the animal would "turn off" the pain stimulus, this might be circumvented by giving a preferred food reward for keeping the stimulus "on" at a given level, as in experiments with shock titration techniques (Kerr, 1982).

6. Use a reward, such as a preferred food, for the correct responses instead of a punisher such as shock for incorrect responses whenever possible (Lea, 1979).

Pain Versus Fear

Any simple analysis of pain is further complicated by recent theoretical and empirical developments which suggest that fear and pain activate quite different kinds of behavior (Bolles & Fanselow, 1980). Rather than being on a continuum, as might seem to be the case intuitively, the evidence shows that fear and pain are associated with fundamentally different motivational systems. The function of fear is one of activating species-specific defensive behaviors, such as freezing, flight, or fighting, which serve to minimize encounters with pain producing stimuli (e.g., predators). Pain, on the other hand, appears to be conducive to the kinds of behaviors which provide for healing and recuperation (e.g., rest, grooming and/or licking the affected area, sleep, etc.).

Bolles and Fanselow (1980) summarize a growing body of evidence which shows that fear takes priority over pain, and that fear can actually inhibit pain under some circumstances (possibly through the release of endogenous opiates). For example, soldiers who are wounded in battle frequently continue fighting and feel no pain from their injuries until after they are removed from the front lines (see Wall, 1979). Likewise, a deer that has been wounded by hunters may flee the scene with defensive behavior indistinguishable from that of uninjured animals. But once the deer is out of danger, pain-related recuperative behaviors predominate.

Food Deprivation

It is important to distinguish between the different methods used to deprive animals of food and the reasons for employing food deprivation. In the vast majority of cases, animals are deprived of food as a means of motivating them to perform various tasks for food reward. It is the nature of their performance on such tasks and not the food deprivation which is the object of study.

Food deprivation is typically applied using one of two procedures. Animals are either deprived of food for a standard period of time (e.g., 24 hours) prior to testing, or animals are maintained at some percentage of their normal body weight (e.g., 80%). There are advantages and disadvantages to both of these procedures. Food deprivation for specified intervals of time has the advantage of being easy to implement, but it fails to take into account species differences in metabolic rates (i.e., 24 hours of food deprivation for a rat is much less severe than it would be for a bird, while for a snake it would be inconsequential). Maintaining animals at a percentage of normal body weight solves this problem, but requires daily handling and deprivation for much longer periods of time to stabilize body weights. In either case it should be noted that the term "deprivation" has come to have some unfortunate connotations. When food deprivation is applied according to a standard time period the most common interval without food is 24 hours (Coile & Miller, 1984). Indeed, 24 hours of food deprivation is precisely what many veterinarians recommend for pets, namely feeding once a day. Likewise, in the case of maintaining animals at some percentage of their normal body weight, experiments rarely involve percentages of less than 80 percent and such levels of sustained deprivation have been demonstrated to actually enhance longevity (e.g., Ross, 1976).

Modifications of and Alternatives to Food Deprivation

The following suggestions have been made as means of reducing or eliminating food deprivation in behavioral research.

1. Water deprivation, which is sometimes used concurrently with food deprivation, should be used to motivate behavior only if thirst or drinking is the object of study. Water deprivation affects an animal's physical condition much more severely than food deprivation because death by dehydration occurs much more rapidly than death by starvation (Lea, 1979).

2. An animal's normal eating pattern should be taken into account when deciding on the duration of food deprivation (see above).

3. In some cases, food deprivation might be avoided by using a highly preferred food as a reward (Lea, 1979).

4. Food deprivation may also be avoided by taking the experiment into the animals living quarters so that it is required to perform for food as and when it wants it, and as such any deprivation would be self-imposed as under natural conditions (Lea, 1979; Moran, 1975). This technique has been used successfully in work on sensory-motor functioning in monkeys (Preilowski, Reger, & Engele, 1984).

Methods of Reducing Pain or Discomfort Due to Experimental Procedures

Anesthesia. General anesthesia should be used instead of local anesthesia for surgical manipulations whenever possible, because it suppresses both pain and fear (Kerr, 1982).

Surgery. Pain-relieving drugs should be administered to animals after surgery whenever this would not interfere with the behavior under study, and animals should be carefully monitored after surgery so that any complications that develop may be treated (Stevens, 1975).

Although spinal transections or decerebration are recommended for surgical experiments when possible, because they render the animal incapable of feeling pain (Kerr, 1982), these techniques are usually of little value in behavioral research since postsurgical assessment requires a relatively intact animal.

Multiple surgeries on the same animal are to be avoided whenever possible because painful consequences may be cumulative (Stevens, 1975).

Euthanasia. Non-recovery experiments on completely anesthetized animals are considered by some to be completely humane (e.g., Russell & Burch, 1959). However, this method is rarely if ever used in behavioral research since most behaviors of interest do not occur when the animal is unconscious and testing is typically conducted postsurgically.

Animals no longer being used in experiments or those that are moribund should be euthanized by one of several humane methods (e.g., barbiturate or anesthetic overdoses, carbon dioxide inhalation, cervical dislocation, or decapitation).

Alternative Physiological Techniques. It has been suggested that in research on the physiological bases of behavior, recording of brain wave patterns should be substituted whenever possible for electrical stimulation, and stimulation of brain areas should be used instead of lesioning or ablating sections of the brain (Lea, 1979). It should be noted, however, that these techniques are not completely interchangeable. Merely recording neuronal firing as an animal behaves allows one to make correlational inferences, not causal ones. If the goal of an experiment is to determine a particular brain area that is responsible for a certain behavior, that area must be manipulated directly. While electrical stimulation of brain areas is useful in establishing causal relationships, the most definitive and reliable results are obtained when stimulation is used in conjunction with lesioning or ablation (e.g., Bennett, 1982).

Alternative Routes of Drug Administration. Most drugs are injected into animals either intraperitoneally, intravenously, intramuscularly, or intracranially through an implanted cannula. Depending upon whether the drug must be given repeatedly over a period of time, the injection procedure can be stressful and/or may cause discomfort.

Within the last decade an alternative method of drug administration has been developed which may replace the need for multiple injections in some chronic drug treatment studies. This method involves the use of silastic capsules which are implanted subcutaneously. These capsules contain the drug which is released slowly into the animal's body and stress produced by repeated injections is, therefore, avoided. The method produces minimal discomfort and is well tolerated by animals (e.g., Ellison, Nielsen & Lyon, 1981). However, since drug absorption rates may differ between individuals, this procedure would not be applicable to studies in which drug dosage must be carefully controlled.

It has also been suggested that aerosols be used to administer drugs instead of injections (Russell & Burch, 1959). Although this technique would seem to hold promise for alleviating the stress produced by injections, it has some major drawbacks. For instance, animals may differ greatly in their inhalation rates and the drug may, instead, be dispersed into the air, preventing adequate control of drug dosage.

Finally, the use of oral administration of drugs would also be preferable to injection, where feasible.

Methods of Reducing Pain or Discomfort Unrelated to Experimental Procedures

Housing. Caging arrangements should consider the natural social structure of the species in question (Lea, 1979). Social animals should be housed in groups whenever possible, and animals that normally lead solitary lives should be housed individually. Subjecting animals that typically live in groups to social isolation, or animals that normally live alone to group housing can be very stressful and may adversely affect experimental results.

Inadvertent stress or discomfort can also be produced by failing to provide an animal with a comfortable resting place in its cage, or by housing normally active species in small cages that limit movement (Stevens, 1975).

Husbandry. Efficient and humane methods of standardized animal care and utilization of qualified animal care personnel can alleviate animal discomfort (Malone, 1982). Strict compliance with federal and state guidelines can also reduce discomfort that animals may be subjected to apart from experimental procedures. Evidence that this is already being accomplished is provided by a recent NIH report which presents the results of a series of site visits to randomly selected institutions (NIH, 1984). The report concludes that reliance on the present NIH policy of voluntary compliance with the provisions of the Animal Welfare Act and NIH policy outlined in the Guide for the Care and Use of Laboratory Animals is an effective way to foster the welfare of laboratory animals.

Managerial Methods of Reducing Pain or Discomfort

Education and Institutional Review Boards. Widespread efforts at educating scientists and students as to humane experimental techniques and animal care methods may alleviate unnecessary pain and discomfort. This might take the form of incorporating into graduate curriculums courses on the ethical treatment of animals as research subjects (Adams, 1982).

It has also been suggested that all universities and research centers establish Institutional Review Boards for animal research, which would be responsible for reviewing all animal research proposals prior to implementation so as to ensure that the procedures comply with humane guidelines (Adams, 1982; Rowan, 1984). In response, the Scientists Center for Animal Welfare in Washington, D.C. has planned a 1984-85 series of educational workshops in cooperation with several universities. The purpose is to explore the state of the art of such committees which are already in existence (e.g., State University of New York at Albany), and to identify essential functions, membership criteria, and procedures. A final consensus conference will be held to develop national guidelines (Laboratory Primate Newsletter, 1984).

Editorial Control. Most journals and professional societies that publish research on animals now have editorial policies and guidelines which require humane treatment of animals (see Appendix 1). For example, the editorial guidelines for the journal Pain state that "We shall refuse to publish any reports where the animal was unable to indicate or arrest the onset of suffering" (Wall, 1975).

Use of Lower Animals as a Substitute for Higher Animals

The use of "lower" animals has been frequently cited (e.g., Bowd, 1980) as a means of circumventing some of the ethical problems associated with the use of animals in research. Why this would be the case, however, has never been made explicit.

In order to put this issue in perspective, first we need to be clear about any distinction that might be used to define "lower" versus "higher". While the distinctions between vertebrate and invertebrate species and warmblooded versus coldblooded animals immediately come to mind, it is important to realize that these are morphological and metabolic distinctions, not behavioral or psychological ones. Indeed, nothing of any cognitive significance has surfaced in the behavioral literature which could be used to differentiate between species as a function simply of whether or not they occupy one or another of these categories. For instance, recent evidence shows that even earthworms, which are invertebrates and coldblooded, still have the apparent capacity to feel pain which may not be fundamentally different from pain sensitivity in seemingly much more complex forms (Alumets et al., 1979). The situation becomes even more garbled and unmanageable in the face of distinctions between harmful versus harmless, pets versus pests, predators versus prey, cute and cuddly versus slippery and slimy and so forth.

If, on the other hand, by lower versus higher one hopes to make distinctions among species based on different dimensions of complexity, it is important to realize that a few invertebrates (e.g., octopus and squid) are at least as complex both anatomically and behaviorally as many vertebrate forms (Sanders, 1975).

Alternatively, many people would like to think they have captured an evolutionary dimension when using the terms higher and lower. It is now clear, however, that such distinctions simply do not apply in any meaningful way to evolution (e.g., Hodos & Campbell, 1969). Among existing species there is no such thing as more or less evolved. Contemporary species are represented by a horizontal, not a vertical dimension. For example, contrary to popular belief, humans did not evolve from chimpanzees. Humans and chimpanzees are related by virtue of sharing a common ancestor (e.g., Yunis & Prakash, 1982). Few, if any, contemporary species are ancestral to one another. In a very real sense, insects are just as evolved as we are (i.e., they have been evolving for an equivalent period of time).

To complicate matters even more, it is becoming increasingly obvious that evolution has not proceeded in a continuous, gradual, linear, or even monotonic fashion. A careful reading of the fossil record shows that evolutionary changes have often been rapid and sporadic, interspersed by varying periods of relative equilibrium (see Eldridge & Gould, 1972; Stanley, 1979). Even different species, as represented by nonoverlapping gene pools, represent fundamentally discontinuous distinctions.

Thus, while it is frequently possible to distinguish between species along biochemical and anatomical lines, as yet we have no meaningful criteria for making such distinctions along psychological or behavioral dimensions. Indeed, there is little or no evidence that familiar distinctions have much in the way of any psychological import. To the extent that there are species differences in pain, suffering, or discomfort, such differences will only be identified as a result of conducting behavioral research on the species in question.

For the time being, where do we draw the line between higher and lower? While it is not clear that there are any failsafe ways of answering this question at the present time (although see Gallup, 1983 for one approach), it is important to re-emphasize that when and if any lines are drawn that they be made on the basis of distinctions which have demonstrable psychological validity. It is increasingly clear that intuition will simply not suffice, nor will most taxonomic distinctions.

It is also important to realize that the use of so-called lower animals as alternatives is predicated on the existence of general principles of behavior which are more or less independent of the species under investigation so that the results will be generalizable. However, it appears that in many instances the behavior of different species is often a unique reflection of the kinds of ecological pressures and selection factors associated with the evolution of the animal in question (e.g., Seligman, 1970).

Thus, depending upon the purpose of the research, the use of alternatives in this instance may require costly validation procedures and detailed studies which ultimately may prove fruitless.

Use of Lower Vertebrates

Aggression. Aggressive interactions between members of the same species have been studied in a variety of fish and reptile species under both laboratory and field conditions. Considerable work has been done on inter-male rivalry among stickleback (Sevenster, 1968) and cichlid fish (Baerends & Baerends-Van Roon, 1950). Inter-male threat behaviors and fighting have also been described in detail for such reptiles as anoles, iguanas, and snakes (see Eible-Eibesfeldt, 1975). Aggressive interactions among lower vertebrates are frequently very stereotyped and species specific. Among stickleback fish, for example, full-fledged attacks can be elicited by a model that is merely the same color as the fish but does not resemble it in shape (see Brown, 1975). In higher vertebrates such as primates, aggressive behavior of one individual may radically influence the behavior of another, and vice versa. Moreover, aggressive interactions among chimpanzees can embody a variety of highly sophisticated introspective social strategies such as deception, grudging, delayed retaliation, reconciliation, etc. (see Gallup, 1983). Thus, it is doubtful that most studies of aggression in lower species could completely substitute for the study of such behavior in higher animals.

Animal Movements. Migration and homing abilities have been intensively studied in several species of fish, particularly the Pacific salmon and eels. Among American and European eels, for example, the eggs hatch in the Sargasso Sea near Bermuda. The larva make a year-long migration toward the coasts of North America and Europe. Upon reaching sexual maturity in seven to 15 years, the adult eels migrate back to the Sargasso Sea to breed (Weisz, 1971).

While migration in fish has been shown to be primarily dependent upon the use of chemical cues in the water (e.g., Eibl-Eibesfeldt, 1975), migration in higher vertebrates, such as birds, is dependent upon a completely different array of abilities. Birds use solar, stellar, and even magnetic cues to navigate. Whales, on the other hand, appear to utilize the topography of the ocean floor and coastline to remain on course for migratory purposes (Gaskin, 1982). Because of such dramatic differences in the way different species respond and perceive the environment it seems doubtful that lower species could be used to replace higher ones for purposes of studying animal movements.

Communication. Visual cues, such as changes in coloration, posture, or body appearance have been shown to be important determinants of social interaction among fish (see Brown, 1975). It is important to note, however, that fish generally have color vision while most mammals do not. Similarly, many fish undergo dramatic changes in appearance, such as flaring of the gill apertures, which have no counterpart in higher animals.

Auditory communication is similarly constrained by species differences. Such communication among amphibians and reptiles, which functions primarily to attract a mate, consists of simple one or two note utterances. Vocalization in higher vertebrates such as birds consist of a wide range and variety of sounds. Moreover, unlike lower species, many birds have to learn their species-specific songs (Manning, 1972). It has also been discovered that many rodents communicate by ultrasonic vocalizations (Leshner, 1978) which have no counterpart in lower animals.

Learning/Memory Mechanisms. While learning has been studied in a variety of diverse species (e.g., Sevenster, 1968; Schwartz & Cogan, 1977), many differences have begun to emerge. Bitterman (1975), for example, has studied learning in goldfish and turtles in an attempt to compare their performance with rats. While there are certainly similarities, there are also a number of distinguishing features separating lower from higher species. For example, rats show a decrement in performance when the accustomed reward is changed, while goldfish and turtles do not. Similarly, rats show resistance to extinction of a learned response which is negatively correlated with reward size, while among goldfish the correlation is positive. The existence of so-called biological boundaries of learning (Seligman, 1970) which appear to have been shaped by unique ecological pressures, also preclude any simple substitution of one species for another in learning paradigms.

Predator-Prey Relations. Prey catching behavior and predator avoidance have been studied in a variety of lower vertebrates such as fish, frogs, and turtles (Eibl-Eibesfeldt, 1975; Huntingford, 1984; Tinbergen, 1951). Because of the similarity across species in behaviors used by prey to avoid predation, it has been suggested that when a general question about reactions to predation (rather than the behavior of a given species) is of interest, invertebrates and lower vertebrates should be used instead of higher vertebrates (Huntingford, 1984). In spite of the apparent similarities, however, there is growing evidence for neurochemical differences underlying similar such behaviors between avians and mammals (see Gallup, Boren, Suarez & Wallnau, 1983).

Unlike prey, predators show vast differences across species when it comes to predator-prey interactions. Frogs, for example, typically sit passively and wait for an insect to come within striking distance. Some carnivores, in contrast, have developed sophisticated hunting strategies that often embody elements of cooperation, and may even culminate in sharing food (see Brown, 1975).

Reproduction/Parental Care. With some notable exceptions (e.g., the African Mouthbreeder fish) parental care of offspring is absent in most lower vertebrates since the eggs are typically abandoned by the parents shortly after fertilization. In contrast, all birds are subject to some type of parental care, and in mammals parent-offspring relations become even more complicated. Similarly, species differences in external versus internal fertilization, seasonal breeding, courtship, pair bonding, and nest building would seem to preclude simple substitutions of one species for another when it comes to the study of reproduction and parental care.

Sensation/Perception. The sensory and perceptual differences between different species are vast. Infrared detection by snakes, echolocation by bats, and the use of web vibrations by spiders renders simple generalizations extremely difficult. The primary mode of prey identification by many snakes is by the use of chemical cues transferred from the tongue to a structure at the roof of the mouth, called Jacobsen's organ. Burghardt (1967, 1970) has shown that among ingestively naive baby snakes there appears to be an innate preference for prey extracts which represent species-typical foods across a variety of different snakes. Each species shows unique attack profiles which appear to be independent of maternal diet, and are not subject to modification by experience (e.g., baby snakes of a species which typically eats minnows but are force fed liver, still show attack responses to minnow extracts but not liver).

In stark contrast with snakes, Galef and Sherry (1973) have shown that baby rats seem predisposed to eat the same diet as their mother, and that the flavor of the maternal milk serves as a medium for the transmission of cues which rat pups use to make their initial food choices. For instance, Gales and Sherry found that by manipulating maternal diet during lactation they could produce corresponding changes in subsequent pup food preferences. Moreover, if rat pups were poisoned in association with another mother's milk they would later avoid the types of food she had been eating.

Spacing Mechanisms. While territoriality is common to a variety of different species, the means by which territories are formed and maintained differ greatly from one species to another. For instance, while birds advertise territorial boundaries by vocalizations, rodents, deer, and many ungulates employ scent marking strategies (Leshner, 1978).

Summary

While the foregoing is hardly an exhaustive or comprehensive account of the behavioral categories listed, it indicates that seemingly simplistic issues associated with the replacement of one species for another in behavioral research raise exceedingly complex questions. Because of diverse adaptations, substitution of lower animals for higher forms may simply not be possible without changing the essence of the phenomenon in question.

CHAPTER 3

USE OF LIVING COMPONENTS

Use of Invertebrates

Although invertebrates comprise 99% of the known animal species, very few behavioral studies use invertebrates as subjects (McConnell & Jacobson, 1973). As a consequence comparatively little is known about invertebrate behavior. However, invertebrates do offer a fertile testing ground for any behavioral theory that claims broad phylogenetic generality (McConnell, 1966). The following is a sample of behaviors studied in invertebrates broken down according to phyla.

Protozoa. Even though protozoa possess both plant and animal-like characteristics and lack nervous tissue, some forms of learning have been demonstrated in the single-cell protozoa Paramecium. Habituation, as defined by the waning of an unconditioned response as a result of repeated elicitation, is considered a primitive form of learning and has been demonstrated in paramecia (Corning, Dyal & Willow, 1973a). Although the results generated much controversy (see Corning et al, 1973a), Gelber (1952) claimed to have trained paramecia to enter a specific area of their water container in order to receive food reinforcement. It has also been reported that paramecia show spontaneous alternation in a T-maze, a phenomenon also observed in rodents (McConnell & Jacobson, 1973).

A recent study of learning ability in paramecia has demonstrated classical conditioning of an escape movement (Hennessey, Rucker, & McDiarmid, 1979). Hennessey et al. also found that paramecia develop memory for the training event, since significantly fewer trials were needed 24 hours later to relearn the response. Data such as these challenge the widely held assumption that learning is a property of synaptic interactions between neurons and not of individual cells themselves.

Arthropods (Insects)

1. Communication, Honeybees recruit others to a new food source by means of a dance that is performed at the hive which conveys distance and directional information (von Frisch, 1967). Many species of insects (e.g., moths, ants) communicate chemically by pheromones, which serve sex attractant, repellant, and/or trail marking functions (see Brown, 1975). Other species, such as the cricket, communicate by songs which are produced by rubbing body parts together (Bennet-Clark, 1970).

2. Navigation. Honeybees have demonstrated extraordinary abilities to locate and return to a food source "mapped out" for them by other bees (see above). They are also capable of returning to an artificial feeding source designed for experimental purposes to test their navigation abilities (Cartwright & Collett, 1983; von Frisch, 1967; Wehner, 1981).

3. Learning and Memory. Habituation has been demonstrated in

a variety of insect species (Corning et al., 1973b). Honeybees also appear capable of more advanced forms of learning such as learning to associate a specific color with a food reward, and they can remember this association after a two-week interval (Wells, 1973). Cockroaches can learn to leave their preferred dark retreats and stay in the light to avoid being shocked (Grier, 1984), while ants have been trained to travel a maze to receive food rewards (Grier, 1984).

4. Behavior Genetics. Because of their simplicity and the relative ease with which their individual genes can be identified, fruit flies have been used extensively to elucidate the genetic basis for a variety of behaviors (e.g., Manning, 1961).

Arthropods (Spiders). Habituation has been demonstrated in a variety of spiders (Corning et al., 1973a), and spiders are capable of learning and remembering the location of prey in their webs (Grier, 1984). They can also be trained to associate food dipped in quinine or sugar with different tones (Corning et al., 1973b).

Echinoderms. Starfish and sea urchins show habituation, and can learn escape behaviors in response to a cue paired with aversive stimulation (Corning et al., 1975).

Annelids. Earthworms show habituation (Corning et al., 1973a), they can learn to associate light with a food reward (Evans, 1968), and they can learn to travel a maze to receive darkness and moisture as the reinforcer (Grier, 1984).

Platyhelminthes. There has been considerable interest in flatworms, since they represent the beginning of primitive bilateral existence. Flatworms show a concentration of nervous tissue and sensory organs in the anterior (head) portion of their bodies, and they have refined internal organ systems (Corning & Kelly, 1973). Flatworms show habituation, they can be conditioned to avoid a light after it has been paired with shock, and they can learn to approach an area for food reward. There are also claims that such learned events are remembered after the planaria undergo regeneration, and that learning can be transferred from one animal to another by cannibalism (see Corning & Kelly, 1973).

Molluscs (Cephalopods). The brain of cephalopods (i.e., octopus and squid) approaches that of the vertebrates in relative size and complexity (Sanders, 1975). Visual discrimination learning has been studied extensively in the octopus. Sutherland (1957) found that they were capable of discriminating between pairs of geometric shapes that differed with respect to vertical, horizontal, and oblique orientations. Squid and octopus show learning performance on a par with mammals on such tasks as detour problems, reversal learning, delayed response, and delayed reinforcement (Sanders, 1975; Wells, 1978).

Summary

Among invertebrates, the only species with neuroanatomy and learning ability comparable to that of vertebrates are the cephalopods. However, practical problems in obtaining, transporting, and housing cephalopods preclude their widespread use as an alternative to behavioral research on vertebrates (Sanders, 1975). Their adaptation to a completely aquatic existence would also make extrapolations to the behavior of terrestrial mammals very difficult and tenuous.

Embryos as Alternatives

Although the use of embryos in behavioral research might seem like an intriguing means of answering evolutionary questions based on the hypothesis that embryonic stages mirror a succession of past evolutionary changes, this has been shown to be untenable (Weisz, 1971). Moreover, evolution does not proceed in a step-wise fashion, building upon the structures already present in one species to form another. Instead, species evolve independently, and as such different phyla do not represent a ladder but rather a phyletic tree (e.g., Hodos & Campbell, 1969).

Embryos have been used in behavioral research as summarized below.

Duck Embryos. Ducklings exposed to maternal calls shortly before hatching show an enhanced response to these calls after hatching, and such findings have been taken as showing that ducklings are capable of imprinting while still in the egg (Gottlieb, 1966). It has also been shown that duck embryos that are devocalized and isolated from other eggs shortly before hatching show a deficiency in selecting a maternal duck call over a maternal chicken call after hatching, indicating that both auditory and vocalization experience prior to hatching are important in the later development of species recognition (Gottlieb, 1975). Duck embryos can also be operantly conditioned. Impekoven (1973) observed an increased rate of foot movements in embryos on the day before hatching when a maternal duck call was made contingent upon foot movements.

Rodent Embryos. Rat and mouse embryos have been used to assess the effects of prenatal stress and hormonal manipulations on behavior occurring after birth (e.g., Leshner, 1978). The most recent focus of interest has been in the area of behavioral teratology, in which animals are exposed to substances such as alcohol or nicotine *in utero* and the effects on behavior are observed postnatally (see Abel, 1982, 1984).

Limitations of Using Embryos

1. Many studies of embryos are conducted when the subject is very close to birth or hatching (e.g., auditory imprinting *in utero*). The advanced developmental status of the organism at this point raises the same kinds of ethical considerations that would apply to the use of animals in general.

2. Embryological studies often involve testing for effects later in adult life (e.g., behavioral teratological studies). Obviously, in these instances embryos are not being used as alternatives since the procedures also require postnatal assessment.

3. In studies involving mammalian embryos, the mother is necessarily involved in most experimental manipulations performed on the embryo. As a consequence, most embryological manipulations on mammals cannot logically avoid the use of adult mammals.

4. Only a very limited number of behaviors can be studied in embryos, partly because of practical problems associated with access to the embryo.

5. Embryos live in a much different environment than fully developed adult animals, which may impose very real limits on the generalizability of effects obtained from embryos.

Plants as Alternatives

In many respects plants are much more similar to animals than commonly recognized. Although plants engage in photosynthetic activity rather than the metabolism that characterizes animals, plants must solve the same fundamental problems associated with adaptation, survival, and reproduction which apply to all species.

From a behavioral perspective, plants differ from animals in two principal ways. Plants lack the means of achieving rapid intraorganism communication and coordination due to the absence of a nervous system. However, plants regulate activities occurring at different sites through the use of hormones. Thus plants and animals do share the basic principles of endocrine function in common. Plants also differ from animals in that they are stationary. Plants must wait for energy to come to them, while most animals are capable of moving about within their environment to obtain different sources of energy.

In spite of these differences, however, plants do show rudimentary forms of "behavior" (e.g., Simon, 1978). Plants can grow and move in response to light, and some plants have achieved the capacity for relatively rapid movement to exploit certain animals as prey (e.g., the venus fly trap). The mimosa plant, which has the capacity to fold its leaves when touched, has been a subject of particular interest. Certain cells of the mimosa plant appear to generate primitive action potentials, which may represent an analogue to neuronal functioning (Sibaoka, 1966). There have also been reports that the folding response of the mimosa plant shows habituation (Applewhite, 1972) and even some of the rudiments of classical conditioning (Armus, 1970). Although there have been some grandiose claims for feelings, emotions, and even thinking in plants based on polygraph recordings (e.g., Tompkins & Bird, 1973), these have been shown to be completely artifactual (Galston & Slayman, 1979).

Because of selective pressure associated with animals that eat plants, a number of plants have developed a variety of predator defenses ranging from thorns and stickers, to toxic chemicals which produce sickness, irritation, or even death if touched or consumed. Recently it has been discovered (see Patrusky, 1983) that plants under attack by insects and microorganisms have developed highly sophisticated defenses involving the emission of antibiotic like substances and chemicals which inhibit insect digestive enzymes. Indeed, some plants can apparently communicate with as yet unaffected neighboring plants to induce leaf chemistry changes in advance of infestation.

In spite of these impressive features of plant life, it is doubtful that plants will ever represent an adequate substitute for or alternative to the use of animals in behavioral research. The lack of a central nervous system and in particular a brain, renders the plant an inappropriate model for studying many, if not most behavioral and psychological problems of interest (cognition, problem-solving, mental illness, etc,).

CHAPTER 4

USE OF NONLIVING SYSTEMS

Nonliving systems such as physical and chemical systems may well be a viable alternative to the use of animals in some selected areas of biomedical and pharmaceutical research. For example, laboratory animals used to be necessary for the assay of fat-soluble vitamins, but now vitamins A, D, and E can be assayed by gas-liquid chromatography and mass spectroscopy (see Rowan, 1984). However, it is unlikely that nonliving systems can ever replace or even reduce the need for animals in behavioral research.

It has been demonstrated that certain chemical reagents show changes in state that oscillate in periodicity and have pacemakers which maintain the rhythm of these state changes (Winfree, 1974). One might argue that such a nonliving analogue might be a useful alternative means of studying circadian rhythms and biological clocks in animals. Yet it must be noted that these chemical reactions are themselves as yet poorly understood (Winfree, 1974), and our current knowledge of the operation and existence of biological rhythms in animals is far from complete.

Tissue Cultures

Tissue culture is the cultivation of single cells or groups of cells outside the body of a living organism. Cell cultures and organ cultures comprise the two main types of tissue cultures. Cell cultures refer to the growth of usually only one cell type at a time. Cell cultures that are derived from an *in vivo* source are primary cultures and have a short life expectancy. Those that have been subcultured over several generations contain either diploid or heteroploid cells and remain relatively stable over long periods of time. Organ cultures are tissues grown in a differentiated, functional state similar to that occurring in an animal's body (see Hegarty, 1972; Dawson, 1975). The main disadvantage of both primary cell cultures and organ cultures for any kind of research is the need for a continuous supply of fresh material from donor animals.

Why Nonliving Systems and Cultures Will Not Suffice

Nonliving systems or isolated parts of living systems will not suffice as an alternative to the use of animals in behavioral research for at least three reasons:

- As previously acknowledged, behavior is a dynamic, emergent process which functions to allow organisms to adapt to moment to moment changes in the environment. In one sense, all behavior ultimately functions to aid and abet survival and reproduction (Daly & Wilson, 1983). Adaptation, survival, and reproduction simply do not apply to cultures or nonliving systems.

- Another distinguishing feature of behavior, as distinct from changes which might occur in tissue cultures and physical systems, is its reliance on information processing. Behavior involves an almost unending series of choices among a vast array of alternatives (McFarland, 1977). While tissue cultures and certain physical systems may indeed change in response to certain environmental events, the nature of such changes does not involve decision making or information processing.

- Behavior is also a byproduct of complex interactions between sensory, neural, hormonal, genetic, and experiential factors. Behavior is not only influenced by the situation at hand, it is also subject to influences which are associated with the developmental history of the organism in question and its prior experience with similar and related situations. Obviously it would be inappropriate to talk about prior experience as it might influence tissue cultures and nonliving systems. Nonliving systems are devoid of the capacity for experience, and as a consequence simply cannot serve as adequate substitutes for or alternatives to the use of live animals.

Computers might appear to be an exception to the foregoing. Indeed, computers do embody information processing and they can be used to simulate decision making. However, the state of the art concerning computer simulation is such that computers will not serve as a viable alternative to the use of animals in behavioral research for the foreseeable future (see section on Computer Simulation). It is also important to note that the related issues of adaptation, survival, and reproduction do not apply to computers and the issue of experience seems to, as yet, transcend the basic principles of computer functioning.

Finally, psychological problems, such as depression, child abuse, anorexia nervosa, catatonic schizophrenia, and suicide are simply not to be found in tissue cultures or computers.

CHAPTER 5

USE OF COMPUTER SOFTWARE

Computer Simulation

Computer simulations differ from other models (e.g., verbal, graphic, mathematical, physical) in an important and fundamental way. These other models are static, that is they model the structure of the system in question, but not its underlying dynamic processes (Greenblat & Uretsky, 1977). A computer simulation is an operating model which not only depicts the state of the system at a particular point in time, but also changes in that system over time. Because dynamic processes are of quintessential importance in behavioral research, computer simulation would appear to be a useful tool for the behavioral scientist.

In behavioral research computer simulations are used in a number of ways. Statistical simulations have been employed with increasing frequency. For example, there is a computer program that simulates random choice behavior in radial arm mazes (Spetch & Wilkie, 1980) and two programs that simulate random movements of animals under different conditions (Bekoff & Wieland, 1982; Cubicciotti, 1982). Thus, the output generated by these kinds of simulations can be compared to animal-generated data to see if factors other than pure chance are influencing the animal's behavior.

Statistical simulations can also be used to test hypotheses that, for one reason or another, are not subject to empirical confirmation. For example, Bennett (1977) employed a computer simulation to test the proposition that "if enough monkeys were allowed to pound away at typewriters for enough time, all the great works of literature would result" (p. 694). Bennett's objective in this instance was also to determine if the extreme cases of human genius could be accounted for through such a process. Thus, it is not merely the output generated by the computer model that is of interest, but the simulation process as well.

Computer simulations also have considerable heuristic value (Estes, 1975). It is often through a simulation that a researcher derives insight about the system or phenomenon being modeled (Freeman, 1971), and as a result the use of computers stimulate additional research. Neches (1982) summarizes the value of computer simulation as a heuristic device as follows: "simulation gives a means of exploring the plausibility of models in which theoretical sophistication exceeds the state of the art in empirical testing....simulations provide tools for empirically analyzing theories in order to better understand their implications and predictions. Simulations are a means of exploring interactions between components of complex models. They pose a practical challenge to operationalize theoretical constructs, which can lead to incidental discoveries about related processes. Finally, they engender a concern with issues of process control that contributes to the development of general principles with broad applications" (p. 78-79, 90).

However, the use of computer simulations as an alternative to the use of animals in research is quite another matter. In order to simulate a living system, you have to have information about that system. The more information at hand, the better the simulation (e.g., Dennett, 1978). In one sense, a completely accurate simulation would presuppose that you knew everything there was to know about the system in question. Moreover, any predictions generated by a simulation must be tested and verified using the system the computer was designed to replace (Mirham, 1976). Finally, simply because the simulation corresponds with what is known about the system, it does not follow that the underlying mechanisms are indeed the same (Reed, 1974). Thus, in a very real sense computer simulations often raise more questions than they answer, which can be very useful for research purposes but seems to preclude their use as viable alternatives to research per se. Then there is also the question of choosing between different simulations. If several simulations of the same system provide roughly comparable results, there is no way to determine with any calculated degree of confidence which model represents the best or most realistic approximation (Estes, 1975).

In terms of future prospects, computers appear to hold some promise for understanding complex cognitive processes. For instance, the computer is often considered analogous, at least in some ways, to the human brain (e.g., Apter, 1970). Both process large amounts of information, and their respective outcomes are a consequence of large numbers of relatively simple operations. On the other hand, there are major differences between computers and brains. Computers have larger and many fewer components than the brain, computer operations occur with much greater speed than neural operations, computers operate through sequential processing of information while brains function in terms of parallel processing, and finally computers attend to all input while the brain is selectively attentive. Such differences, however, do not preclude the use of computers as functional models (Pylyshyn, 1978). Moreover, computers carry the advantage of being demanding of rigorous and exact description, and one need not invoke a variety of hypothetical or mentalistic (e.g., hope, fear, desire, intention) variables to describe their functioning (Reed, 1974).

One of the most promising prospects for computer simulation lies in its use as an instructional tool (Lehman, 1980; Ruggiero, 1977). Not only can computer simulations be used as an alternative to the use of animals for instructional purposes in some instances, but simulations also allow students greater participation in the learning process (Greenblat & Uretsky, 1977).

For research purposes, however, computers function primarily as an adjunct or a tool and not an alternative. In addition to those already noted, Neches (1982) lists several other disadvantages of computer simulation: "(1) A formal specification of a model need not imply a comprehensive presentation. Since programs are rarely presented in full with accompanying documentation, we remain dependent on verbal descriptions of the model. This can raise problems in determining whether the program performs as it does for the reasons claimed by its author....(2) Programs frequently involve simplifying assumptions in order to facilitate implementation. These simplifications, however, cause the program to diverge from the theory it supposedly represents. (3) Programs can be written to work only for a restricted set of examples.... In the absence of some analysis of the formal properties of the domain, there is no automatic guarantee that the examples presented are representative of the domain or that the principles required to handle a given set of examples are sufficient to account for the entire domain. (4) The inputs or data base for the program can be structured in ways that simplify its task, but which are not necessarily psychologically plausible....(5) Data or procedures supplied to the program to define different examples for it to handle may, in fact, constitute nonnumerical parameters that give the program considerable flexibility in fitting psychological data....(6) The programmer may hold back data or procedures that would have confused the program had they been available. That is, the program may appear to perform well, not because it has the capacity to choose the correct action from all possibilities, but rather, because the difficult choices are not offered to it" (p. 78).

Table 3

Examples of Current Attempts at Computer Simulation of Behavioral Phenomena

Behavioral Category	Source	Simulation
Spacing mechanisms and animal movements	Bekoff & Wieland (1982)	space use and movement patterns
	Cubiciotti (1982)	space use and movement patterns
	Jovellanos & Gaskin (1983)	movements of juvenile Atlantic herring
	Murai, Thompson & Wellington (1979)	animal spacing
	Peterson (1980)	mosquito flight patterns
	Pyke (1981)	honeyeater foraging
	Spetch & Wilkie (1980)	random choice in radial arm mazes
Reproduction, parental care	Freeman & McFarland (1974)	copulatory behavior of the male rat
	Hausfater, Aref & Cairns (1982)	infanticide in langurs
	Knowlton (1979)	evolution of reproductive synchrony
	Symmons & Rosenberg (1978)	mating behavior of *Spodoptera littoralis*
	Toates & O'Rourke (1978)	sexual behavior in male rats
Learning, memory, problem solving	Barto & Sutton (1982)	anticipatory responses in classical conditioning
	Benedict (1979)	classical conditioning
	Goude (1981)	behavior in a two-valenced psychoecological space

	Salu (1983)	learning in neural systems
	Stanley (1976)	habituation
	Woodward & Bitterman (1976)	mechanisms for reducing inhibition
Communication	Dobson & Lemon (1979)	bird song
	Dooling, Clark, Miller & Bunnell (1982)	animal vocalizations
	Shiovitz & Lemon (1980)	bird song
Neural function	Lee & Marudarajan (1982)	neuron models
	Minamitani & Hagita (1981)	neural basis for pain and touch
Bodily maintenance	Barnwell & Stafford (1975)	food intake
	Toates (1974)	homeostatic control of drinking behavior
Sensation and perception	Barlow, Narasimhan & Rosenfeld (1972)	visual pattern analysis
	Cartwright & Collett (1983)	landmark learning by bees
	Jaffe (1980)	chemical recruitment in ants

Advantages and Disadvantages of Computer Simulation

One of the chief benefits of modeling techniques using computer simulation is the refinement of actual experiments to be conducted on animals (Carson, 1983). Rather than an alternative to the use of animals in behavioral research, computer simulation is an adjunct to such research. Indeed, several authorities (e.g., Guyton, 1981; Newton, 1975) feel that the use of computers will actually serve to increase rather than decrease the use of animals in research because the data can be analyzed much faster and in much greater detail, thus leading to proportionately more problems to investigate . In this sense computers serve to facilitate research, not substitute for it.

The use of computers as an alternative to animals is best summarized by Kerr (1982). "At the present time and for the foreseeable future it seems clear that the computer will not be a feasible substitute for experiments on animals. The fundamental reason is that a computer cannot acquire data other than those that are generated by carefully designed experimental studies in animals. What the computer does provide is a superb technique for processing vast amounts of data with great speed and accuracy and for presenting them in almost any manner the investigator desires. To suggest that enough data are already available from previous work, so that from them programs can be generated and subjected to a variety of permutations that would lead to new insights, overlooks an important fact. In any animal experiment there are numerous variables over which we have little control, and there are virtually always as many more about which we as yet know nothing but which may have very significant influences on the phenomenon under investigation. In real life, which after all is what matters in biologic research, these variables may be crucial and may give important clues to entirely unsuspected phenomena that are sometimes far more important than the original subject of the study. In a word, computers do not generate new concepts or acquire new data. They process data and permit the investigator to view it in more manageable or novel ways, and this may facilitate new hypotheses or insights." (p. 21-22).

To construct a computer simulation which would completely substitute for the use of live organisms in behavioral research would require that we knew everything there was to know about the behavior in question, which in turn would preclude the need for a computer simulation for research purposes. To proceed on the basis of incomplete information would necessarily mean that the results would be rendered incomplete. Moreover, to the extent that research represents an attempt to expand, refine, and enlarge the data base any novel output from the computer program would have to be checked against the animal it was meant to replace, thus creating the need for additional research with animals rather than precluding it.

CHAPTER 6

VALIDATION OF ALTERNATIVES

Since we expect uncompromising rigor and precision from scientists, it only follows that scientists should have every reason to expect the same from any would-be alternative to the use of animals in research. According to Rowan (1984), "any alternative system must provide data which leads to the same conclusion with at least the same degree of confidence as that obtained from the system being replaced" (p. 260).

With this in mind, it is important to distinguish between the use of alternatives for assessment and screening on the one hand, and research on the other. Unlike screening techniques (e.g., LD-50, the Draize test), the objective of research is to enlarge and elaborate our understanding of basic phenomena. The name of the game when it comes to research is discovery, refinement, and replication. In contrast, once a screening technique has been developed and tested any further work is unnecessary, while in the case of research the answer to one set of questions invariably leads to a host of other questions and the need for additional research.

Thus, when it comes to research the issue of alternatives raises a peculiar paradox. To the extent that Rowan's definition of an alternative system applies, it means that each and every new finding derived from the alternative would have to be checked against the system it was meant to replace. Obviously this would simply introduce another step in the research process without reducing the need for the original system. If an alternative (living or otherwise) could be devised which provided what would appear to be an invariant fit between itself and all of the existing data previously derived from the system it was designed to replace, then, and only then, could one feel reasonably confident of finding a correspondence between any new data generated by the alternative and what could have been expected from the animal that had been replaced. However, even under such ideal conditions, prudence would still seem to demand spot checks involving the original system at many steps. Since research is a quest for new information, there is never any guarantee that new information derived from an alternative will be exactly isomorphic with data that might have been obtained from the original system.

When it comes to assessment, behavioral tests are being used with increased frequency by the pharmaceutical industry for purposes of drug screening (e.g., Riley & Meyer, 1984). While acceptable alternatives have been developed for the Draize test, it is clear that tissue cultures and bacteria will simply not suffice when it comes to behavior. This is particularly true in light of the fact that behavior now appears to be one of the most sensitive means of detecting adverse effects of low level teratogens and other toxicological agents. Behavior is an extremely complex function of neurological, neurochemical, endocrinological, genetic, and experiential factors and therein lies its unique advantage as a screening and assessment device. Any alternative which involves a system which is less complex than the animal it is designed to replace may, for that reason alone, be inadequate. Indeed, this logic would suggest that when it comes to sensitivity for screening and assessment purposes perhaps we ought to be replacing lower species with higher ones, e.g., witness the thalidomide tragedy which could have been avoided had appropriate testing been performed with primates rather than rodents (see Gallup & Suarez, 1980a).

Not unrelated to the question of validation are the topics of availability and feasibility. In many instances the replacement of one species for another may simply not be practical because of problems related to the maintenance and availability of the replacement (e.g., octopus), or because of financial and technical problems associated with having to retool equipment and laboratory facilities to obtain comparable data.

Finally, it is important to note that there simply have not been many viable or productive suggestions about alternatives to the use of animals in behavioral research by persons writing on the topic of alternatives. For instance, in a recent and well balanced treatment of the issues concerning the use of animals in research, Rowan (1984) devotes only 19 out of 286 pages of text to the use of animals in behavioral research. Likewise, in a book by Smyth (1978) entitled "Alternatives to Animal Experiments", only 2 out of 218 pages deal with animals in behavioral work. Lastly, in the widely respected Bibliography of Bioethics (Walters, 1975-80, 1981-83) there are no entries concerning alternatives to the use of animals in behavioral research.

CHAPTER 7

CONSEQUENCES OF DISCONTINUING BEHAVIORAL RESEARCH ON ANIMALS

If behavioral research on animals were to stop it would have a number of severe consequences. Among other things it would mean having to foreclose the possibility of many further advances in the prevention and cure of a variety of psychological and biomedical problems which are simply not amenable to research by any other means. It would also mean foregoing further developments in which the behavior of animals can have far reaching implications for ecological problems and would provide sound solutions to environmental problems which preclude the use of toxic pesticides. It would also preclude research which has the clear potential to benefit the animals themselves, and would either retard or prevent continuing work on a variety of basic processes involving questions of brain function, genetics, hormonal influences, learning, motivation, cognition, and aggression.

As this report has shown, at the present time there do not appear to be many, indeed any, viable alternatives to the use of animals in many areas of behavioral research. While the social and economic consequences of restricting such research are difficult to estimate, one way to begin to realize the importance of behavioral research is to examine the magnitude of mental and emotional problems in the human population at large in this country. It is commonly estimated that one out of every ten people living in the U.S. will at some time be institutionalized because of a serious psychological problem (e.g., Coleman et al., 1980). More people suffer from psychological problems than all other major forms of illness combined. The economic consequences, in terms of lost productivity, crime, personal injury, drug abuse, law enforcement, and institutionalization, run into the billions and billions of dollars. For instance according to a recent report released in June 1984 by the Research Triangle Institute (Maser, 1984), the direct and indirect costs to society of alcohol, drug abuse, and mental illness in the calendar year 1980 was approximately 190.7 billion dollars. To the extent that behavioral research with animals might lead, as it already has, to methods for dealing with alleviating such problems the consequences of discontinuing such work could be considerable, not to mention the needless pain and suffering that people with mental and emotional problems would have to continue to endure. In light of such considerations, some scientists have concluded that it would be ethically irresponsible not to do behavioral research with animals (e.g., Marcuse & Pear, 1979; Seligman, 1975).

Finally, it is important to note in this context that there is no way in advance of determining with complete certainty which experiments will produce important findings and which will not. Indeed, Gallistel (1981) makes a compelling case that any reduction in the number of experiments conducted with animals would produce a corresponding reduction in valuable data (see Figure 1). It is also often the case in science that what were considered important findings at the time turn out to be dubious in retrospect, while those which were seen as being insignificant later exert profound influences (see Gallup & Suarez, 1980 a).

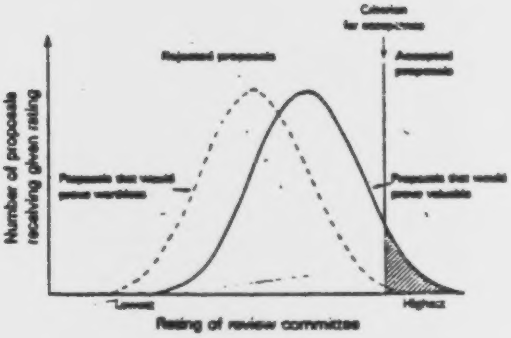

Figure 1. The dilemma faced by review committees that are determined to eliminate worthless experiments. The shaded area represents the proposals that are allowed to be carried out. Even though the criterion for acceptance has been set so high as to eliminate most of the research that if carried out would have proved valuable, some research projects that in fact prove worthless are still done. Before the fact, many worthless experiments look as good as or better than many very valuable experiments. The number of worthless experiments permitted becomes negligible only when the criterion is so high that nearly all the valuable experiments are rejected (from Gallistel, 1981).

CHAPTER 8

CONCLUSIONS

1. Naturalistic observations and field studies while providing useful sources of preliminary information, are not viable alternatives to laboratory investigations of animal behavior (p. 6-7).

2. There are many instances in which behavioral research on animals has important biomedical implications, and in the last analysis any hard and fast distinctions between behavioral and biomedical research are not possible (p. 8).

3. There are a number of important reasons why animals are used in behavioral research, which include questions of control, objectivity, developmental and genetic issues, methodology, complexity, the ideal case, heuristics, practical applications, and ethical considerations (p. 9-12).

4. Generalizations about categories of behavior (e.g., parental care, migration, communication) in unrelated species are frequently misleading since different species often evolved independently and achieve superficially similar results by fundamentally different means (p. 13-14, 59-68).

5. There are some behavioral phenomena which are found in so few species that substitution of species is not possible (p. 13-14).

6. The use of animal models is the only practical way of investigating many behavioral phenomena (p. 15-16).

7. Behavioral research with animals has frequently produced tangible benefits for the animals themselves (p. 17-19).

8. Behavioral scientists have often been more reliant on the use of some species as opposed to others. There are many reasons why particular species are used in behavioral research, and much of the research already involves the use of "lower" animals (p. 21-22).

9. Because of public health considerations there is growing evidence for an increased reliance on the use of animal behavior as a means of screening low level teratogens and other suspected toxicological agents (p. 26-27).

10. Through a more judicious choice of experimental designs and statistical techniques it might be possible to effect a marginal reduction in the number of animals used in some instances of behavioral research (p. 30-37).

11. There is no evidence that the use of "lower" species will reduce the problems of pain and suffering in behavioral research (p. 42).

12. Preconceived notions of suffering are often at odds with actual preferences shown by animals (p. 42-44).

13. Some animals have evolved in such a way that the absence of stress may have deleterious physical and psychological consequences (p. 43).

14. Of all the types of pain producing stimuli, electric shock represents the ideal and most humane technique in most cases (p. 45).

15. The degree of pain and suffering endured by animals in behavioral research is of a much lower magnitude and frequency than has often been asserted, and often less than what animals endure under natural conditions (p. 43, 46-47).

16. It is not at all clear what can be gained by substituting lower species for higher species in behavioral research. Indeed, as yet there are no meaningful behavioral or psychological criteria for distinguishing between lower and higher species (p. 59-62).

17. Vast species differences in behavior compound and confound many attempts at substitution of one species for another (p. 63-68).

18. The use of embryos as alternatives is of limited value at best, and in many instances may actually exaggerate the problems associated with the use of animals in behavioral research (p. 75).

19. Nonliving systems and cultures are not viable alternatives to the use of animals in behavioral research (p. 81-82).

20. Computer simulation may actually increase the need for animals in behavioral research (p. 91).

21. The use of computer models as a substitute for animals in behavioral research may be a logical impossibility (p. 91-92).

22. The use of computer simulations for teaching purposes could reduce the number of animals used in instructional settings (p. 86).

23. The use of animal behavior as an assessment and screening device is fundamentally different from its use for research (p. 93-95).

24. The use of any alternative may simply introduce another step into the research process without having any appreciable influence on the need for animals in behavioral research (p. 93-94).

25. Effective screening and assessment based on behavior requires the use of higher rather than lower species (p. 95).

26. Were behavioral research on animals to stop it would have vast social, psychological, environmental, biomedical, and economic consequences (p. 97-98).

27. Any attempt to limit behavioral research with animals will impose a corresponding limit on significant findings and important developments (p. 98-100).

28. Much of the current behavioral research with animals is noninvasive, and in many instances (e.g., social behavior, animal movements, communication, habitat and food selection, exploration, reproduction and parental care) involves little or no pain, distress, or discomfort.

29. A concern for the humane treatment of laboratory animals is consistent with good science. Obviously, data obtained from animals in good health, which are adequately cared for, and maintained under comfortable conditions, are preferable and ultimately may be more generalizable.

30. Scientists themselves have often taken the initiative in alleviating pain in experimental animals and have devoted entire books to the subject (e.g., Kitchell & Erickson, 1983).

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IV. CARE AND USE OF LABORATORY PRIMATES

Report of the Committee on Research and Development - June 1, 1984

Claud A. Bramblett, Chairman

The primary purpose of this report is to promote high standards of ethical behavior toward animal subjects and improve the general welfare of nonhuman primates in the laboratory. We recognize that the use of laboratory primates provides a unique data base for treatment of human problems as well as advancing knowledge in general. However, the committee takes the position (c.f., Simmonds, 1973) that a baseline of good health and an unstressed state produces more credible and accurate experimental results in laboratory animals. It is particularly important that graduate and advanced students become familiar with basic principles of laboratory primate management, husbandry, and care. We hope that this report, sanctioned by the ASP as the legitimate voice of professional and concerned primatologists, is an informative supplement to existing general guidelines and regulations as they apply to primates.

Research protocols of related activities with primates are required to conform to the procedures described in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act. More detailed treatment of management, handling, are listed in the reference section of this report. The concerns and requirements of ASP are, in the judgement of this committee, best addressed by the 1980 Institute of Laboratory Animal Resources (ILAR) Revision (ILAR News, 22, 1980). This document reviews basic husbandry and management information, including quarantine and conditioning procedures, disease prevention, testing and control. This report specifically addresses the care of nonhuman primates and supplements the more general Guide for the Care and Use of Laboratory Animals, Revised 1978.

Guidelines for shipment of animals were developed by the Interagency Primate Steering Committee (1981, 1982). Investigators are expected to study and adhere to this document prior to shipping any nonhuman primate. The document outlines health measures prior to shipping, administrative factors involved in shipping, details of container construction, container size, problems related to temperature, and husbandry matters (food, water, bedding, minimization of potential stress).

Given the social propensities of primates and their rapidly decreasing numbers in natural habitats, the Committee considers group housing, preferably breeding groups, to be the most desirable and least stressful housing choice. In certain circumstances, such as when individual animals must be tested elsewhere on a regular basis, single housing is preferable to group housing since culling the target animal from the group might be very stressful for all members of the group. When experimental protocols require single subject primates, it may be desirable to allow animals visual contact with one another to increase the probability of behavioral interaction. Physical contact may be allowed in these conditions if the animals are carefully supervised to prevent fighting and its attendant risk of injury. It is important to recognize that damage to an animal from improper housing may be psychosocial as well as physical.

Group housing pens allow for more freedom of movement and provide opportunities for expression of social behavior. Social companions are the most complex and stimulating element in the stable laboratory environment. Primate groups that are allowed to become reasonably stable in group pens equipped with resting perches, nesting boxes, and escape barriers, as appropriate, should provide excellent rearing environments and high quality, reproductively capable offspring (Erwin and Denni, p. 24, in Erwin et al., 1979).

Group formation is potentially hazardous. Species appropriate age-sex classes of potential members must be considered when establishing groups. Erwin et al. (1979) and Goodwin and Coelho (1982) review some aspects of primate aggressive behavior relating to group constitution, organization and disruption in captivity. These factors should all be carefully considered by any scientist planning to establish a colony of nonhuman primates.

Advice of primatologists with established colonies should be sought to minimize the possibility of social stress and physical trauma in the monkeys that will populate the new colony.

The committee recommends that ILAR guidelines be followed as a universal standard. In addition the Committee makes the following specific recommendations and statements of general principles.

1. Facility Veterinarian

ASP acknowledges the responsibility of every facility conducting primate research to employ an attending veterinarian, charged with assuring appropriate treatment of animals and their maintenance in good health (Department of Agriculture Regulations, Chapter 9, May, 1972). Programs of disease control and prevention, euthanasia, and adequate veterinary care shall be established with the supervision and assistance of a doctor of veterinary medicine.

2. Animal Studies Committee - Protocol Review

Each facility/institution conducting primate research shall establish an on-site Animal Studies Committee. The committee shall review exprimental protocols to assure proper care of animals, consistent with research need and the species utilized. Appropriately staffed Animal Studies Committees shall consist of at least the attending veterinarian, two or more scientists, and a lay person.

3. The Use of Chair-Restraint

A. Chronic restraint of primates in chairs, i.e., continuous chair restraint exceeding 1 day, must be scientifically justified, as demonstrated by approval of the scientific merit of the project by peer review (Animal Studies Committee). Animals are never to be chaired merely for the convenience of the investigator.

B. The attending veterinarian is responsible for day to day decisions concerning procedures and the health of animals.

C. Primates should be evaluated for their ability to adapt to chair restraint. Protocols such as the one used by the University of Washington's Regional Primate Research Center, Seattle, WA 98195, should be adhered to during such evaluation periods. If the decision to chair animals is made, repeated careful examinations by the attending veterinarian shall be conducted and the veterinarian's advice about the primate medical condition while living in the chair should be followed.

D. Careful consideration of alternatives (behavioral modification, tethering,...) to chair restraint is strongly encouraged.

4. The following basic principles of laboratory management are emphasized by the Committee on Reseach and Development:

A. A knowledge of the habits of primates is a prerequisite for the procurement, shipment, quarantine, and handling of animals to maintain healthy research specimens.

B. Animals shall be provided with adequate space, housing, and equipment conforming to federal, state, and local laws and regulations. Animals shall be provided with adequate space for normal postural adjustments and exercise.

C. Only animals that are lawfully acquired shall be used in laboratories. Newly arrived animals shall be segregated by species, placed in quarantine rooms, and appropriately housed. Laboratories shall conduct active health monitoring programs.

D. Cages should be designed to facilitate sanitary conditions. Cages shall be devoid of sharp edges or corners that can cause injury to the animal and the handler.

E. Design of laboratory buildings shall take cognizance of problems relating to environmental factors such as: ventilation, temperature, humidity, photoperiod, noise, exposure to other animals, and security.

F. When escape from primary enclosures is possible, animals should be confined to secured areas. A closed door shall remain between animals and the outside world when animals are being handled or examined.

G. All unnecessary persons shall be restricted from animal areas. Windows provided for viewing by visitors contribute to the safety and health of both animals and man, minimizing transmission of disease and parasites.

H. Unless experimental design dictates otherwise, diets should be available for extended periods. Clear, fresh water should be available at all times. diet and water containers should be constructed to provide easy access, while preventing urinary and fecal contamination. Open watering pans are undersirable. Nutrition influences many aspects of health and behavior. Otherwise well-designed experiments may be questioned if satisfactory nutritional status of the experimental animals cannot be assured.

I. Investigators shall use appropriate anesthetic, chemical, or drug techniques. When anesthetics, chemicals, or drugs will not be used in experiments requiring discomfort or other stressors, investigators shall provide satisfactory justification for the procedure to the Animal Studies committee and to the Facility Veterinarian.

J. Given the recognized need for both conservation and production of nonhuman primates, animals shall be recycled between laboratories.

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GUIDING PRINCIPLES FOR RESEARCH INVOLVING HUMANS AND ANIMALS

BYLAWS OF THE SOCIETY FOR NEUROSCIENCE

Recommendations from the Declaration of Helsinki

I. Basic Principles

1. Clinical research must conform to the moral and scientific principles that justify medical research and should be based on laboratory and animal experiments or other scientifically established facts.

2. Clinical research should be conducted only by scientifically qualified persons and under the supervision of a qualified medical man.

3. Clinical research cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.

4. Every clinical research project should be preceded by careful assessment of inherent risks in comparison to foreseeable benefits to the subject or to others.

5. Special caution should be exercised by the doctor in performing clinical research in which the personality of the subject is liable to be altered by drugs or experimental procedure.

II. Clinical Research Combined with Professional Care

1. In the treatment of the sick person, the doct must be free to use a new therapeutic measure, if his judgment it offers hope of saving life, reestablishi health, or alleviating suffering.

If at all possible, consistent with patient psycholog the doctor should obtain the patient's freely give consent after the patient has been given a full explanatio In case of legal incapacity, consent should also b procured from the legal guardian; in case of physic incapacity the permission of the legal guardian replac that of the patient.

2. The doctor can combine clinical research wi professional care, the objective being the acquisition new medical knowledge, only to the extent that clinic research is justified by its therapeutic value for th patient.

III. Non-Therapeutic Clinical Research

1. In the purely scientific application of clinic research carried out on a human being, it is the du

of the doctor to remain the protector of the life and health of that person on whom clinical research is being carried out.

2. The nature, the purpose and the risk of clinical research must be explained to the subject by the doctor.

3a. Clinical research on a human being cannot be undertaken without his free consent after he has been informed; if he is legally incompetent, the consent of the legal guardian should be procured.

3b. The subject of clinical research should be in such a mental, physical and legal state as to be able to exercise fully his power of choice.

3c. Consent should, as a rule, be obtained in writing. However, the responsibility for clinical research always remains with the research worker; it never falls on the subject even after consent is obtained.

4a. The investigator must respect the right of each individual to safeguard his personal integrity, especially if the subject is in a dependent relationship to the investigator.

4b. At any time during the course of clinical research the subject or his guardian should be free to withdraw permission for research to be continued.

The investigator or the investigating team should discontinue the research if in his or their judgment, it may, if continued, be harmful to the individual.

Guiding Principles in the Care and Use of Animals

Approved 1980 by the Council of The American Physiological Society

Animal experiments are to be undertaken only with the purpose of advancing knowledge. Consideration should be given to the appropriateness of experimental procedures, species of animals used, and number of animals required.

Only animals that are lawfully acquired shall be used in this laboratory, and their retention and use shall be in every case in compliance with federal, state and local laws and regulations, and in accordance with the *NIH Guide*.

Animals in the laboratory must receive every consideration for their comfort; they must be properly housed, fed, and their surroundings kept in a sanitary condition.

Appropriate anesthetics must be used to eliminate sensibility to pain during surgical procedures. Where recovery from anesthesia is necessary during the study, acceptable technic to minimize pain must be followed. Muscle relaxants or paralytics are not anesthetics and they should not be used alone for surgical restraint. They may be used for surgery in conjunction with drugs known to produce adequate analgesia. Where use of anesthetics would negate the results of the experiment such procedures should be carried out in strict accordance with the *NIH Guide*. If the study requires the death of the animal, the animal must be killed in a humane manner at the conclusion of the observations.

The postoperative care of animals shall be such as to minimize discomfort and pain, and in any case shall be equivalent to accepted practices in schools of Veterinary Medicine.

When animals are used by students for their education or the advancement of science such work shall be under the direct supervision of an experienced teacher or investigator. The rules for the care of such animals must be the same as for animals used for research.

Principles for Use of Animals

Appendix 1. *NIH Guide for Grants and Contracts*, Vol. 7, No. 17, November 10, 1978

The Personnel

1. Experiments involving live, vertebrate animals and the procurement of tissues from living animals for research must be performed by, or under the immediate supervision of, a qualified biological, behavioral, or medical scientist.

2. The housing, care, and feeding of all experimental animals must be supervised by a properly qualified veterinarian or other scientist competent in such matters.

The Research

3. The research should be such as to yield fruitful results for the good of society and not random or unnecessary in nature.

4. The experiment should be based on knowledge of the disease or problem under study and so designed that the anticipated results will justify its performance.

5. Statistical analysis, mathematical models, or *in vitro* biological systems should be used when appropriate to complement animal experiments and to reduce numbers of animals used.

6. The experiment should be conducted so as to avoid all unnecessary suffering and injury to the animals.

7. The scientist in charge of the experiment must be prepared to terminate it whenever he/she believes that its continuation may result in unnecessary injury or suffering to the animals.

8. If the experiment or procedure is likely to cause greater discomfort than that attending anesthetization, the animals must first be rendered incapable of perceiving

pain and be maintained in that condition until the experiment or procedure is ended. The only exception to this guideline should be in those cases where the anesthetization would defeat the purpose of the experiment and data cannot be obtained by any other humane procedure. Such procedures must be carefully supervised by the principal investigator or other qualified senior scientist.

9. Post-experimental care of animals must be such as to minimize discomfort and the consequences of any disability resulting from the experiment, in accordance with acceptable practices in veterinary medicine.

10. If it is necessary to kill an experimental animal, this must be accomplished in a humane manner, i.e., in such a way as to insure immediate death in accordance with procedures approved by an institutional committee. *No animal shall be discarded until death is certain.*

The Facilities

11. Standards for the construction and use of housing, service, and surgical facilities should meet those described in the publication, *Guide for the Care and Use* *Laboratory Animals*, DHEW No. (NIH) 80-23, or otherwise required by the U.S. Department of Agricult regulations established under the terms of the Laborat Animal Welfare Act (P.L. 89-544) as amended 19 and 1976 (P.L. 91-579 and P.L. 94-279).

Transportation

12. Transportation of animals must be in acc with applicable standards and regulations, espec those intended to reduce discomfort, stress to t animals, or spread of disease. All animals being recei for use as experimental subjects and having arrived the terminal of a common carrier, must be promp picked up and delivered, uncrated, and placed acceptable permanent facilities.

ANIMAL BEHAVIOR SOCIETY

ABS ANIMAL CARE GUIDELINES

I. FEDERAL, STATE, AND LOCAL REGULATIONS.

Members, their students, and research associates have an obligation to become thoroughly familiar with, and comply with the provisions of the Animal Welfare Act (1970), the Endangered Species Act (1973), 62 Statute 686 (1948), and the Migratory Bird Treaty Act (1918), all subsequent amendments, rules, and regulations persuant to these Acts, and other applicable federal, state, and local regulations to which the researcher may be subject. In the case of animals trapped in foreign countries, researchers have an obligation to become familiar with, and comply with the relevant regulations of those countries.

II. STANDARDS OF RELATED ORGANIZATIONS.

Members and their affiliates should attempt to become familiar with standards of organizations, other than the ABS, which relate to the care, treatment, and use of animals in behavioral research.

III. ABS GUIDELINES FOR ANIMAL CARE.

A. Choice of Animals.

1. Species. The species chosen for study should be well-suited to answer the questions posed. When the research involves the use of procedures which are likely to cause unavoidable pain or discomfort to the animal, and when alternate species can be used, the researcher should employ the species which in his/her opinion, is least likely to suffer.

2. Number of individuals. In the case of 1) laboratory experiments involving non-optimal conditions (e.g. pain or discomfrot), or 2) field studies involving manipulations potentially detrimental to the population, the researcher should utilize the smallest number of animals necessary to accomplish the research goals.

3. Status of natural populations. If the taxon or population is endangered or threatened, individuals should not be collected except in cooperation with a serious conservation endeavor and in compliance with the Endangered Species Act (1973) and other pertinent legislation. The researcher is responsible for ascertaining the status of the taxon or population in question and should uphold not only the letter but also the spirit of those laws dealing with endangered species both in the U.S.A. and in the foreign countries where the animals originate.

B. Observation of Natural Populations.

Observation of free-living animals in their natural habitat may involve disruption, particularly if feeding, trapping, or marking is involved. While field studies may further scientific knowledge and advance awareness of human responsibility towards animal life, the investigator should always weigh any potential gain in knowledge against the adverse consequences of disruption for the animals used as subjects and also for other animals in the ecosystem.

C. Procurement of Animals.

Researchers should obtain animals only from reliable and legal sources. As much as possible, the researcher should ensure that those responsible for handling of the animals en route to the research facility provide adequate food, water, ventilation, and space, and do not impose undue stress. If animals are field-trapped this should be done legally and in a manner which is as painless and humane as possible.

D. Housing and Animal Care.

Unless contraindicated by research protocol, animals should be maintained and cared for in such a manner as to meet their species-typical and individual needs. Minimal standards are specified in *Guide for the Care and Use of Laboratory Animals*, DHEW Publications No. (NIH) 74-23.

E. Pain or Discomfort.

Since traumatic effects of experimental procedures are likely to vary from species to species, the researcher should attempt to ascertain how much his/her research species is likely to suffer as a result of the procedures employed. In general, researchers are urged to consider the use of alternative procedures before employing techniques which are likely to cause physical or psychological discomfort to the animal. In accordance with the existing animal welfare laws, pain or discomfort, even when unavoidable, should be minimized to the greatest extent possible under the requirements of the experimental design. Attention should be given to proper pre- and post-operative care in order to minimize preparatory stress and residual effects.

(continued...)

E. **Pain or Discomfort.** (continued)
Unless specifically contraindicated by the experimental design, procedures which are likely to cause pain or discomfort should be performed only on animals which have been adequately anesthetized.

F. Final Disposition of Animals.

Whenever practical or feasible, researchers should attempt to distribute their animals to collegues for further study. However, if animals are distributed for use in additional experiments, care should be taken that the same animals are not used repeatedly in experiments which involve invasive surgical procedures or other treatments which are likely to be stressful or painful. Except as prohibited or prescribed by federal, state or local laws, researchers may release field-trapped animals if this is practical and feasible, and if it is critical to conservation efforts. However, the researcher should consider that releases into the wild may be injurious or detrimental to existing populations in the area, and releases should be made only after the researcher has determined that the negative impact is likely to be minimal. As a general guideline, field-trapped animals should be released only within the same area where they were trapped (unless conservation efforts dictate otherwise), and only when their ability to survive in nature has not been impaired, and when they do not constitute a health or ecological hazard to existing populations. If animals must be destroyed subsequent to the study, this should be done by euthanization in as humane and painless a way as possible, and death of the animals should be confirmed before bodies are discarded.

PRINCIPLES FOR THE CARE AND USE OF ANIMALS

Approved September 3, 1979 by the Council of Representatives, American Psychological Association

An investigator of animal behavior strives to advance our understanding of basic behavioral principles and to contribute to the improvement of human health and welfare. In seeking these ends, the investigator should ensure the welfare of the animals and should treat them humanely. Laws and regulations notwithstanding, the animal's immediate protection depends upon the scientist's own conscience. For this reason, the American Psychological Association has adopted the following Principles to guide individuals in their use of animals in research, teaching, and practical applications. All research conducted by members of the American Psychological Association or published in its journals must conform to these Principles.

1. The acquisition, care, use, and disposal of all animals shall be in compliance with current federal, state or provincial, and local laws and regulations.
2. A scientist trained in research methods and experienced in the care of laboratory animals shall closely supervise all procedures involving animals and be responsible for insuring appropriate consideration of their comfort, health, and humane treatment.
3. Scientists shall insure that all individuals using animals under their supervision have received explicit instruction in experimental methods and in the care, maintenance, and handling of the species being used. Responsibilities and activities of individuals shall be consistent with their respective competencies.
4. Scientists shall make every effort to minimize discomfort, illness, and pain to the animals. A procedure subjecting animals to pain, stress, or privation shall be used only when an alternative procedure is unavailable and the goal is justified by its prospective scientific, educational, or applied value. Surgical procedures shall be performed under appropriate anesthesia; techniques to avoid infection and minimize pain must be followed during and after surgery. Euthanasia shall be prompt and humane.
5. Investigators are strongly urged to consult with the Committee on Animal Research and Experimentation at any stage preparatory to or during a research project for advice about the appropriateness of research procedures or ethical issues related to experiments involving animals. Concerned individuals with any questions concerning adherence to the *Principles* should consult with the Committee.
6. Apparent violations of these *Principles* shall be reported immediately to the facility supervisor whose signature appears below:

Name: ______________________________

Position: ______________________ Phone: ______________

If a satisfactory resolution is not achieved, a report should be made to the responsible institutional authority designated below:

Name: ______________________________

Position: ______________________ Phone: ______________

Unresolved allegations of serious or repeated violations should be referred to the APA Committee on Animal Research and Experimentation.

7. These *Principles* shall be conspicuously posted in every laboratory, teaching facility, and applied setting where animals are being used. All persons in each laboratory, classroom, or applied facility shall indicate by signature and date on the attached sheet that they have read these *Principles*.

Committee on Animal Research
and Experimentation
Board of Scientific Affairs
American Psychological Association
1200 Seventeenth Street, N.W.
Washington, D.C. 20036

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ALTERNATIVES TO ANIMAL USE IN VETERINARY MEDICINE

October 31, 1984

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INTRODUCTION

The Veterinarian's Oath

"Being admitted to the profession of veterinary medicine, I solemnly swear to use my scientific knowledge and skills for the benefit of society through the protection of animal health, the relief of animal suffering, the conservation of livestock resources, the promotion of public health, and the advancement of medical knowledge.

I will practice my profession conscientiously, with dignity, and in keeping with the principles of veterinary medical ethics.

I accept as a lifelong obligation the continual improvements of my professional knowledge and competence."

Adopted by the American
Veterinary Medical Association
July 1969

Veterinary medicine is the discipline which has the primary responsibility for maintaining and improving the health and well-being of animals. Professionally speaking, veterinary research addresses normal structure and function, the causes, diagnosis, prevention and treatment of disease in clinical and experimental animals. Generally, the profession concentrates on health of animals important for food and fiber, and on companion animals. Research on food and fiber producing animals is important in order to continue to utilize plant and animal resources for human sustenance. The rapidly increasing world populations will require maxim production efficiency from both plants and animals in the future (Bywater, 1980). Veterinary research must play a prominent role in controlling those diseases of importance to food producing animals and man. Other species receiving veterinary attention include laboratory animals, fish and aquatic animals, zoological and wild animals. Veterinary medicine addresses, conservatively speaking, 30 of the different animal species of economic, ecologic, and environmental importance. Veterinary research encompasses studies on domestic animals including cats, cattle, chickens, dogs, donkeys, goats, horses, sheep and turkeys; laboratory animals such as guinea pigs, hamsters, mice and rats; non-human primates such as baboons, new world monkeys and old world monkeys; exotic birds such as parakeets, parrots, cocatiels, cocatoos, etc.; fresh water and marine fish; birds of prey such as falcons, hawks, eagles; deer, antelope, elk; lion, tigers, elephants, llamas, snakes, and marine mammals among others. The ultimate goal of veterinary research is the prevention and alleviation of disease and suffering in animals.

Fewer animals are utilized for veterinary research as opposed to the numbers used for toxicological testing of drugs and chemicals. Furthermore, the nature of research on animals in veterinary medicine generally addresses different types of questions than those required for testing programs.

It is important to note that reasonable numbers of animals used in experiments must be sufficient to provide valid results. The numbers of animals used in experiments depends on the desired confidence levels (95 percent) needed to interpret the results and to minimize the variation among animals in response to the treatments. Variation among animals is often difficult to predict without actually doing the experiment; however, in some cases, variation among animals can be estimated from past experiments (Race, 1977). When this information is known, the minimum number of animals needed to give 95 percent confidence in the results can be calculated. Future reductions may be possible, but numbers used at present are near the minimum.

More sophisticated experimental designs may allow reduction of numbers (Newton, 1977). One method is sequential analysis in which experiments are done on one animal at a time until sufficient data has been gathered to allow 95 percent confidence in the results. This method has pronounced disadvantages in that the variation in control animals must be known very precisely and it would require that many more animals to used. Also, the time to complete many experiments would become unacceptably long (Armitage, 1975).

Veterinary researchers, like many other researchers over the last three decades, have emphasized the use of rigorous scientific methods to study diseases in tightly controlled, isolated systems. This emphasis has resulted in a major reduction in the use of whole animals, whereas alternative methods such as *in vitro* cell and organ cultures, substitution of lower vertebrate species, improved anesthetics, and surgical procedures, as well as computer simulated models have been and are continuing to be developed for research purposes. The vast majority of veterinary researchers have been actively seeking research approaches which can be appropriately substituted for the use of whole live animals in experiments in keeping with their commitments and obligation to "The Veterinarian's Oath".

Veterinary researchers are responsible for addressing anatomical, physiological and disease complexities in a diverse array of animal species spanning invertebrates (e.g. oysters) to large mammals (e.g. horses, elephants). In order to accomplish the required research goals, it is necessary to carry out research in intact animals of lesser quality within the same species. Body functions consist of millions of actions and reactions. Each is dependent upon many others, and it is highly unlikely that man will ever be able to simulate all of the complexities of the body with alternate methods (Davidson, 1977). Diseases involving the rumen can only be studied in ruminants, and repair of fractures in horses can only be satisfactorily studied in horses. Research on animal illnesses sometimes reveal diseases similar or identical to those affecting man. In some instances, these animal models such as simian acquired immune deficiency are the only models that can provide the basic means of studying and understanding treatment regimes for both animal and human disease(s).

The current status of the alternative use of animals in veterinary research is addressed in this chapter. Although it is impossible to cover all aspects of veterinary research, the use of current technology is described and representative examples are included when appropriate.

VETERINARY RESEARCH SUMMARY MATRIX

Discipline:	Continued Use of Animals	Continued but Modified Use of Whole Animals	Use of Animal Components	Use of Nonliving Systems	Use of Computer Programs
Anesthesiology	+	+/-	+/-	+/-	+/-
Animal Genetics & Breeding	+	-	+/-	+	+/-
Behavioral Biology	+	+/-	0	0	+/-
Biochemistry & Biophysics	+	+	+	+	+/-
Cancer Biology	+	+/-	+	0	+/-
Entomology	+	+/-	+/-	0	-
Epidemiology	+	0	0	0	+
Immunology	+	+/-	+	0	+/-
Microbiology	+	+/-	+	+/-	+/-
Molecular Biology	+	+	+	+	+/-

Key:		
	+	Yes
	+/-	Possible
	-	Doubtful
	0	Not possible

	Continued Use of Animals	Continued but Modified Use of Whole Animals	Use of Animal Components	Use of Nonliving Systems	Use of Computer Programs
Nutrition & Metabolic Diseases	+	+/-	+/-	-	+/-
Parasitology	+	+/-	+/-	+/-	+/-
Pathology	+	+/-	+/-	+/-	+/-
Pharmacology	+	+/-	+/-	+/-	+/-
Physiology	+	+/-	+/-	+/-	+/-
Radiology/Radiation Biology	+	+/-	+/-	+/-	+/-
Surgery	+	+	+/-	-	+/-
Toxicology	+	+	+	+/-	+/-
Virology	+	+	+	+/-	+/-

Anesthesiology

Statement: Basic aspects of anesthesiology deal with chemical, physical and physiologic interactions in the body. Applied aspects of anesthesiology deal with chemical alterations of the central and the peripheral nervous system with an ultimate goal of complete cessation of pain but intact physiologic function.

Continued Use of Animals. It is essential to test new anesthetic agents in animal species because each species may have its own idiosyncrasies. For example: physiological differences in respiratory systems and the abilities to detoxify anesthetics vary in different species. Anatomical and physiological characteristics of respiratory systems in animal species differ, i.e., birds have lungs and air sacs unlike mammals which have only lungs; large mammals (over 200 kg) have because of significant hydrostatic forces, marked mismatching of ventilation and perfusion which can significantly compromise respiration (Soma, 1977).

Similarily, the detoxification of drugs (anesthetics) is varied among species (viz. certain birds {parakeets} are extremely sensitive to the local anesthetic lidocaine; cats are unable to glucuronide conjugate morphine derivative drugs and as a result cats are highly susceptible to morphine and oxymorphone, resulting in toxicity. In contrast, morphine and it's analogues are completely ineffective in ruminants and must be dosed in extremely high levels to observe a short acting effect (Jones, 1977). Another anesthetic, xylazine, is significantly more potent in ruminants than monogastrics.

Continued but Modified Use of Whole Animals. Lower vertebrates and invertebrates are essential to understand anesthetic-animal interaction, and for the relief of pain in the utilization of those lower animals in basic and applied research protocols. Basic studies on many anesthetics are carried out using lower vertebrates before applying the anesthetic agents to domestic species. Mice, rats, guinea pigs, and rabbits are frequently utilized to evaluate anesthetic potency and compare to known anesthetic agents before the products are administered to higher mammals.

Use of Animal Components. Local effects on the primary target organ (nervous tissue) and detoxification or functional studies (kidney, liver, heart) can be made using animal components employing an *in vitro* whole organ or cell culture protocol.

Use of Non-living Systems. Non-living systems have little use in the study of anesthesiology at this time. Eventually, these systems could be used to evaluate aerosolized particles carrying inhalation anesthetics. Artificial membranes may also be used to evaluate membrane transport of anesthetics.

Use of Computer Programs. Computer programs for research purposes in anesthesiology are not extensively utilized at present. There are numerous examples of computer programs which can be applied in anesthesiology research. The programs deal more specifically with the pharmacokinetics (i.e., absorption, distribution, metabolism and excretion) of the anesthetics. Other programs deal with acid/base and ventilation/perfusion ratios. However, many, if not all pharmacokinetic studies are theories; the only way to verify such modeling studies is with animal testing. The same exists for acid/base and ventilation/perfusion programs. The development of new programs and the employment of existing programs is not anticipated soon because few, if any, veterinary anesthesiologists have the background and training (i.e., computer programming, pharmocokinetics) to apply it to their research protocols.

Animal Genetics, Breeding and Reproduction

Statement: These disciplines cover the broad aspects of genetic characteristics of animals, reproduction and reproductive cycles, embryos and fetal development in normal and disease states, and natural and artificial breeding including insemination and embryo manipulations.

Continued Use of Animals. Each of the domestic species which veterinary medicine addresses has its own unique genetic constitution, reproductive characteristics and breeding characteristics. As a result there are no good "model" systems that can be substituted for studying the unique characteristics other than using animals within the species. Examples of characteristics which differ in species include size of animals, reproductive and breeding processes, diseases of the reproductive tract, length of pregnancy, and types of placentation (Cole, 1980; Gillespie, 1981; Jubb, 1970).

Research on animals must continue in order to preserve and exploit desirable germplasm. These studies are necessary to increase animal production efficiency and to minimize or eliminate exotic or transplacentally transfered infectious diseases (Council, 1984). It has been shown that cows infected with bluetongue, akabane and bovine virus diarrhea can be bred and the collected embryos successfully transferred to non-infected recipients without transferring the infection (Bowen, 1983; Singh, 1982). This technology can now be used to break the chain of infection in infected strains of animals.

Continued but Modified Use of Whole Animals. Since there are unique characteristics in each species, the number of animals used in future experiments will not be greatly reduced because there are no suitable substitutes. Genetic studies require relatively large numbers of animals in order to draw definitive conclusions in complex polygenic systems of outbred species. In order to verify genetic traits such as production or disease resistant traits, progeny must be followed and evaluated. This can only be evaluated through arranged breedings with individuals with other known genetic backgrounds (Cole, 1980). No pain is associated with these studies.

Improved technology is being applied in all areas. For instance, the early embryo transfer procedures required modest surgical intervention to recover embryos. In the last few years, non-surgical techniques have been developed to harvest embryos which can then be transferred to recipient animals.

Substitution of lower mammals is not probable for studies on population and production genetics. Mice are used to refine technical approaches to manipulative genetics before applying the technology to domestic species. Micro-manipulation such as embryo splitting is practical in mice before using on domestic animals. Transfection of genetic material into embryos is done with mice embryos before being carried out in cattle, sheep, etc. Studies on reproductive cycles are carried out for the most part in the domestic species. This is not likely to change because the estrous cycles, pregnancies, and embryonic and fetal development are unique to each species. Suitable models have not been identified.

Use of Animal Components. Recent advances in culture techniques have led to increased use of *in vitro* culturing of ova, embryos and organs. Ova and embryos can be harvested by non-surgical means. Cultured abattoir specimens are used for *in vitro* studies of hormone production. For instance, ovaries can be put in organ cultures for studies on the physiology and hormone production (Chubb, 1979).

Use of Non-living Systems. Non-living systems are being used to assay hormone levels in blood and secretions, such as milk, in studies defining hormonal patterns during estrous cycles. Enzyme-linked immunosorbent assays and radioimmune assays are the principle means of measuring hormones (Munro, 1984).

Use of Computer Programs. Current utilization of computer programs as simulated models for breeding and reproduction is minimal. Computer programs are now being applied to genetic studies and will be used more frequently as more genetic markers are identified. Serotyping of bovine lymphocytes to identify histocompatibility markers is analyzed by computer programs (Mickey, 1980).

Behavioral Biology

Statement: Behavioral biology is the study of observable responses of the organism to given circumstances and the inter-relationships between the organism and these circumstances.

Continued Use of Animals. Because behavioral biology is the study of the responses of an animal to given circumstances, the use of whole animals is essential to this field of study (Hind, 1970). To study or observe how an animal acts (behaves) or conducts itself in a particular manner requires this whole animal. Examples such as the study of age at castration of male horses and the effect on aggressive behavior require that the behavioral responses of the castrated animal be observed under varying environmental conditions over time.

Continued but Modified Use of Whole Animals. Reduction in the numbers of animals required for a given set of experiments may be possible by extrapolation of data from closely related animal species. Such extrapolation may lead to more tightly controlled experiments with sharper focus on specific quetions. The end result of this extrapolation and sharper focus should be a reduction in the number of experiments required and a subsequent reduction in the number of animals used. Multidisciplinary approaches to behavioral responses may yield more rapid advances in acquiring knowledge. For example, the correlation of hormonal levels and behavioral responses has led to a better understanding of reproductive behavior.

Interventive behavioral research which is likely to produce pain should be moderated by analgesics, providing the substances used do not affect the behavior being studied.

Substitution of lower mammals can be used to gain greater understanding of basic behavioral responses; however, there is no substitute for the particular species under study. For example, one does not substitute mice to study the maternal behavior of range cattle.

Lower vertebrates and invertebrates are of little use as substitutes for the study of behavioral responses of higher mammals because their responses are not as complex nor do they interact socially as do the higher mammals.

Use of Animal Components. Because behavioral biology requires the whole animal for observation, use of cell, tissue, or organ cultures is of limited or no use.

Use of Non-living Systems. Because behavioral biology requires the whole animal for observation, use of non-living systems is of limited use.

Use of Computer Programs. The use of computer programs should assist in the analysis of the volume of data acquired in behavioral observations. More data can be acquired and analyzed, leading to more rapid acquisition of knowledge. This results in the more efficient use of animals because more questions can be answered per given set of experiments. The end result is a reduction in the number of animals used over time. In addition, hypotheses may be tested using computer modeling. The direct result of this can lead to a reduction in the number of observations and, subsequently, the number of animals used.

Biochemistry and Biophysics

Statement: These disciplines are perhaps the most basic of biological sciences as they are concerned with identifying the fundamental chemical and physical processes of living organisms. These disciplines may be considered as the interface between the biological and the physical sciences. The disciplines encompass processes such as chemical reactions within cells (especially enzymatically catalyzed reactions), the molecular and atomic structure of cell components, and the physical properties (strength, viscosity, elasticity, melting point, etc.) of cellular and noncellular (plasma, bone, etc.) components. These disciplines are useful in the study of basic disease processes as well as healthy animals.

Continued Use of Whole Animals. Because they are so basic, dealing with the physics and chemistry of biomolecules, biochemistry and biophysics probably use fewer whole animals than any other discipline in the biological sciences. However, there are many instances when the results of *in vitro* research must be confirmed in live animals. For example, an enzyme may be studied extensively in a test tube and its properties defined with regard to the effect of adding known inhibitors or activators. But the biochemical milieu within the body is still not completely defined, and there may be unknown inhibitors and activators requiring in vivo studies to determine the properties of enzymes in situ.

Continued but Modified Use of Whole Animals.

Reduction of insult. More extensive use of blood-sampling catheters would make repeated sampling with needles unnecessary in many, but not all, biochemical investigations. General anesthetics are not feasible with long-term experiments and may interfere with many short term experiments.

In some biochemical and biophysical research, surgical procedures are necessary. For example, to study the effect of various treatments on the structure of bile salts and bile pigments, catheters must be surgically implanted in the bile ducts of animals to obtain samples. Generally though, most of the experiments in animal biochemistry and biophysics use tissues obtained from dead animals.

Lower vertebrates and invertebrates can be substituted for domestic animals in some circumstances. Once the complete molecular structure of a biochemical substance is known for two species, and if the substance has a similar structure in the two species, it is often feasible to substitute one species for another for in vivo research.

Even though there are structural similarities between substances in two species, the cellular environment in which the substances exist may differ markedly between the species, and the *in vivo* properties may differ accordingly. Also, the same substance may exist in different parts of the cell in different species and therefore, have different *in vivo* properties. For example, the enzyme phosphoenolpyruvate carboxykinase, which is a liver enzyme involved in the production of glucose, exists only within the cell mitochondria in chickens, only outside the mitochondria in rats, and both inside and outside the mitochondria in cattle. The *in vivo* properties of this enzyme could be expected to be (and are) quite different in the three species (Ballard, 1971).

Lower animals can substitute higher mammals for preliminary experiments and for screening of large numbers of compounds or treatments that might have biochemical or biophysical effects. Ultimately, any experiments with promising results will have to be repeated in higher mammals.

Use of Animal Components. Tissue components can be and are used extensively in biochemistry and biophysics. This trend is not likely to diminish.

Animal cell cultures are used and will continue to be used extensively. Many chemicals and physical parameters that influence metabolic pathways can be studied successfully in cultured cells. Most of the important results must be verified in intact animals because there can be interactions in intact animals that cannot be observed or predicted from results from tissue cultures. Also, much work with animal tissue cultures must be done with freshly harvested cells because the cells of many organs cannot be cultured through very many generations, or cells may change radically with each succeeding generation.

Cell cultures have the advantage that the cells from one animal can be divided into many small lots which allows many observations to be gathered from one animal.

Invertebrates, microorganisms, and plant classes of life forms have been useful in investigating some of the most basic aspects of biochemistry and biophysics. Much that has been learned about the genetic code, for example, was learned from microorganisms. Lower life forms are also sources of compounds that are useful in experiments with higher organisms. These life forms have great limitations, though, as substitutes for mammals. For example, even though the genetic code is nearly identical for bacteria and mammals, the molecular apparatus that transcribes and translates the code into proteins is quite different (Watson, 1976).

Use of Non-living Systems.

Physical systems. One example of a physical system that has substituted for some animal use is the use of artificial cell membranes. Such membranes can be made with synthetic compounds (e.g., lipids synthesized from petroleum) although the use of compounds of animal origin is common. These membranes have provided basic information about the passage of compounds through membranes and about the conduction of electrical impulses along membranes (Chang, 1972). Most of these experiments do not substitute for experiments in whole animals because most of these experiments could not be performed with whole animals. Significant results which might have an impact on animal health must be verified or coordinated with results of animal experiments.

Chemical systems. A large amount of biochemistry research is done with isolated biochemical compounds. Fundamental research on the structure of body substances and the chemical effects of body substances on each other is performed in this way. It is now possible to chemically synthesize many body proteins and metabolic compounds, which means they can be used without requiring animals. There is reason to hope that in the future, in vitro protein synthesis will be cheaper and enzymes and other proteins can be studied without killing animals. Advances in tissue culture, chemical synthesis, and mammal to bacteria gene transfer techniques should make pure mammalian proteins available. However, most body proteins still cannot be made synthetically because we do not know their structure, and the only way to study them is in live animals or to extract them from animal tissues. The study of biochemical changes associated with disease will still require the use of animals with induced diseases as sources from which abnormal biochemicals can be isolated for study.

Use of Computer Programs. Computer programs are used extensively in biochemistry and biophysics, a trend that will increase. Programs can be used to model complex chemical kinetic systems. Once the properties of the enzymes that comprise metabolic pathways are known, it is possible to investigate the effects of varying amounts of intermediates on the rate at which the product is produced at the end of the pathway. A big problem with this approach is that the properties of most enzymes are not completely known nor do we know all of the effects that various substances in cells can have on enzymes (Dixon, 1979; Segel, 1975).

Cancer Biology

Statement: Cancer biology is the study of all factors relating to the cause, development and subsequent behavior of tumors (cancer). The goal is to obtain information leading to improved methods for prevention or treatment and cure of cancer.

Continued Use of Animals. Discrete steps in the multifactorial development of tumors can often be best analyzed in *in vitro* systems, but the complex interplay of genetic, immunological, endocrinological, nutritional and other factors which influence the outcome of carcinogenesis can only be fully investigated in whole animals. For instance, tumors unique to animal species under the influence of hormones secreted by endocrines include the mixed mammary tumors of dogs and tumors of the pituitary pars intermedia in horses (Moulton, 1978). These associations can only be studied in these species. The interrelationships between the major histocompatibility complex gene markers for susceptibility or resistance to neoplastic diseases can only be studied in the species in which the tumors occur. The associations have been observed in chickens with the B genotype with Marek's disease and the BoLA-W8 in leukosis of cattle (Lewin, 1984; Shat, 1982). Basic studies on feline leukemia infection and to some extent simian acquired immune deficiency (SAIDS) has provided background information for the current research on human acquired immune deficiency syndrome (Essex, 1979; Hause, 1981; Hunt, 1983; Osborn, 1984; Trainin, 1982). Some neoplasms are unique to a species and can only be studied in that same species. The same holds true for studies of new or improved methods for treating cancer (Theilen, 1979). Some neoplasms such as transmissible venereal tumors of dogs, pulmonary adenomatosis of sheep, and mixed mammary tumors of dogs are unique to species and can only be studied in them (Moulton, 1979). The same holds true for studies of new or improved methods for treating cancer because the pharmacology of chemotherapeutic agents may be different in each animal species (Theilen, 1979). Immunotherapy must be tailored to the species in which the therapy is to be used. This is required for doseage determination and to assess the efficacy of the product.

Continued but Modified Use of Whole Animals. Modified use of whole animals might be possible if techniques of molecular/ cellular biology enable more sharply focused studies in whole animals.

Where there is experimental production of cancer, the alleviation of pain by measures used in clinical cases can be considered, provided it is not likely to affect the processes being studied. Euthanasia can be employed when the appropriate endpoint for a particular study has been reached or when it appears that the animal is suffering undue discomfort.

Substitution of lower mammals (e.g., laboratory rodents) is already being practiced to the extent possible. Since there are important interspecies differences in the biology of cancer, study (or at least validation) is needed in the animal species of concern. For instance, mammary tumors in dogs tend to be mixed tissue tumors which are very different from those occurring in laboratory animals (Moulton, 1979).

Lower vertebrates and non-vertebrates are important sources of clues as to the fundamental causes and mechanisms in cancer biology. Basic studies are carried out on lymphosarcomas in walleye and hepatomas in trout (Roberts, 1978).

Use of Animal Components. As in other areas of biomedical research, many of the basic molecular and cellular interactions involved are being investigated in cell, tissue and organ culture. Studies on the molecular basis of carcinogenesis are carried out in cell cultures as are studies on the feasibility of using different chemotherapeutic regimes on avian retroviruses. As methods continue to develop, more research will be carried out in these cell, tissue and organ cultures.

Use of Non-living Systems. At present, there is very little use of non-living systems in cancer research in veterinary medicine. This will increase as soon as sufficient information becomes available to synthesize membranes and then study the role of chemicals, antibodies, etc.

Use of Computer Program. Computer programs have been used for epidemiological studies of animal neoplasms. Presently there is little evidence that computer program models have been developed for veterinary cancer biology. It is likely that a few models will be developed in the next decade. Before the models can be proven successful, it will require the use of additional animal experiments to prove that the model(s) are valid.

Entomology

Statement: Entomology is the study of insects and arachnids (mites and spiders). Relatively small numbers of mammals are used in the study of entomology, but where mammals are used in the study of entomology the mammals play an extremely important role.

Entomology is important to veterinary medicine because many arthropods are important parasites and vectors of animal diseases. Arthropods and the diseases they either induce directly or spread from animal to animal (and man to man) are major causes of human and animal suffering worldwide. Diseases of food animals transmitted by or caused by arthropods include:

- Bluetongue virus
- Scabies
- Texas tick fever
- Q fever
- Louping ill
- Anthrax
- Screw worm infestation
- Spirochetosis
- Louse infestation
- Anemia
- Dermatitis
- Rocky Mountain spotted fever
- Heartwater
- Tularemia
- Trypanosomiasis
- Nasal bot infestation
- Cattle warbles
- Sheep ked infestation

Diseases of companion animals transmitted or caused by arthropods include:

- Western equine encephalitis
- Flea allergies
- Plague
- Erlichiosis
- Piroplasmosis
- Louse infestation
- Demodectic and sarcoptic mange
- Heartworms
- Tick paralysis
- Tapeworm infestation
- Equine infectious anemia

Continued Use of Animals. Research in entomology of importance to veterinary medicine is aimed at controlling populations of noxious arthropods, controlling or preventing the disease entities they cause, and studying the relationship of parasite and host (Beesley, 1982).

The relationship of a particular parasite to a particular host is often quite specific and not amenable to the substitution of even another mammal (Arthur, 1983). For example, to study the host-parasite relationship of fleas and dogs, one must use dogs (Baker, 1983). No other model is likely to provide satisfactory in developing more effective ways of controlling fleas or more effective ways of treating flea-induced skin conditions in dogs.

In many cases, the behavior of the insect or mite on the host is an area of investigation (Hafez, 1982). The selection of a particular host, the exact site on the host chosen to feed, and the method of feeding of the parasite are important to an understanding of both parasite control and disease transmission. Such studies can require an intact host mammal.

Diseases produced by insect or mite infestations are often immunologically mediated. In some cases the host immune response can overcome the infestation; in other cases, it is the host's immune response that causes the disease. A phenonemon as complex as a host's allergic reaction to a parasite can only be studied in an intact animal. (Brown, Antibody...1983; Brown, Immune...1982; Brown, Rhipicephalus...1983; Fisher, 1983; Stone, 1983; Woldehiwet, 1982).

In other cases, the mammals used are nct directly a part of the research. In these cases, the mammals may be used as a food source for the parasite so that the parasite can be studied in the laboratory. Small colonies of mice, guinea pigs, rabbits, and other mammals are often maintained in entomology laboratories to be bitten by mosquitoes, ticks, and other arthropods. In some cases, the arthropod pest cannot be reared any other way (the pests nutritional or behavioral requirements may not be understood well enough to allow a substitute food source). In other cases the arthropods can be fed on drawn blood from the same laboratory animals, or on various blood-containing artificial media. In the latter instance, the whole animal is still present in the laboratory (as a blood donor), but the number of animals required is reduced (Adham, 1982; Barnard, 1982; Young, 1983).

Another indirect way that mammals are used in the study of entomology is as sources of immunologic reagents. Many laboratory studies make use of the antibodies to either parts of the parasite or parts of the host. For example, it is possible to determine what host species a particular population of mosquitoes is feeding on by using immunologic tests on stomach contents of wild-caught mosquitoes. To evaluate the potential effectiveness of a particular predator as a biological control for mosquitoes or other pests, it is possible to perform immunologic tests on gut contents of the carnivore to determine if it has in fact been eating the pest in question. In both the tests described above, mammals such as rabbits, mice, and guinea pigs would have been used to produce the antibodies used in the immunological tests (Washino, 1983).

Continued but Modified Use of Whole Animals. The numbers of animals used as sources of blood for feeding mosquitoes, ticks, fleas, lice, and other arthropods are very small and therefore reductions in this area of study are not likely to have a great impact on the number of animals used in research. It is already standard practice to use the lowest host phylogenetically that is acceptable to the arthropod. This is also an area where the use of animals is not particularly stressful for the mammal (a mouse, for example, is usually anesthetized, placed in an enclosure with mosquitoes, and then removed and allowed to recover after the mosquitoes have finished feeding).

In studies of the host-parasite relationship, it would usually be necessary to use both the parasite and the target host in order to yield relevant information about the natural disease process. Studies of a parasite on an unnatural host may or may not yield useful information. Substitution of lower animals would frequently be impossible.

Use of Animal Components. Animal components are used with great regularity. The immunologic tests referred to earlier use sera derived from antibody-producing animals. These products augment and enhance studies, though, rather than to replace animals used in other contexts. The net effect of serologic studies is to increase the numbers of animals used, since the antibodies are usually used in contexts where animals might not be used otherwise (as in the studies on mosquito stomach contents described above).

Animal components and byproducts can also be used as part of the culture media for some arthropods. For example, milk byproducts such as casein can be a part of the media used to raise fly larvae. Certain blood-sucking arthropods can be cultured on drawn blood or on partially synthetic media containing blood. This yields a net reduction in the number of animals required (Howarth, 1983; Munderloh, 1982; Paine, 1983).

Use of Non-living Systems. Many studies, such as purely anatomical studies, might involve only the insect (Binnington, 1983; Pinkerton, 1983; Roshdy, 1982). A researcher might use electron microscopy to visualize a parasite within the insect vector (Anderson, 1983; Clerx, 1983; Kocan, 1983). Studies of insect and arachnid vectors might involve trapping and studying the vector apart from the mammalian host (Sacks, 1983). The population density of a vector in a particular habitat might be studied (Beier, 1983). While many entomological studies do not involve using a mammalian host, these studies could not properly be said to be substituting for a study in which mammals might have been used. Instead, the studies not involving mammals represent qualitatively different types of studies. In most studies of veterinary importance in which a mammal was used, no non-living system could be said to be a satisfactory substitute.

Use of Computer Program. Computers are useful in the compilation of data following a large study (Artsob, 1983; Crane, 1983; Grayson, 1983). Mathematical models can be used to study the effects of population dynamics on the incidence of disease (Smith, 1983). Conceivably, they could be used to make climatological predictions as to years and geographic areas in which population booms of arthropod pests could occur. While these uses of the computer are important to the study of entomology, it is also true that none of these uses of the computer replace animals (since laboratory animals were not used in these studies to begin with). It is unlikely that any computer program could substitute in a meaningful way for animals in veterinary studies presently requiring animals (antibody production, sources of nutrients, host-parasite relationship).

Epidemiology

Statement: Epidemiology is the study of patterns of health and disease in populations of animals. This study frequently focuses on the effect of specific parameters such as infectious diseases, nutrition, chronic diseases or environmental factors. Epidemiology is also both positive and negative in that it studies factors leading to good health as well as factors leading to poor health or disease (Mausener, 1974).

Epidemiology has two phases. The descriptive or observational phase deals with the monitoring or surveillance of populations of animals. The experimental phase of epidemiology develops hypotheses realized from field studies or the descriptive phase.

Continued Use of Animals. Because epidemiology is the study of populations of animals the continued use of animals is essential to this discipline.

Continued but Modified Use of Whole Animals. Reduction in the numbers of animals under study may be accomplished by properly designed experiments. In descriptive epidemiology, however, this discipline studies naturally-occurring patterns of health and disease. By definition then, there is no direct control over the numbers of animals involved in the process being observed. In experimental epidemiology, a reduction in the numbers of animals being used can be attained by more carefully designed experiments where the least number of animals which yield significant results are used.

Induced pain is essentially non-existent in most epidemiological studies.

Substitution of lower mammals is commonly practiced in the pursuit of experimental epidemiological studies. For example, if possible, mice are substituted for natural populations while the study is in development. However, ultimately, field trials using natural populations are required. Descriptive epidemiology does not allow the substitution of lower mammals.

Lower vertebrates and nonvertebrates are not commonly used in place of higher mammals.

Use of Animal Components. Epidemiology is the study of populations of animals. Disciplines working upon epidemiological problems may use components such as those seen in parasitology and virology, but epidemiology itself requires the animal population.

Use of Non-living Systems. The same generalizations as stated above apply for the use of non-living systems.

Use of Computer Programs. In descriptive epidemiology, the use of the computer allows for rapid analysis of data using sophisticated statistical software. This advances knowledge. Experimental epidemiology assesses and verifies risk factors identified during the course of field studies. If enough data are obtained during the course of the descriptive phase, computer modeling can be performed to predict the results of the changing variables. Ultimately, however, field trials have to be conducted to validate the model.

Immunology

Statement: Immunology is the study of an individual's ability to respond with cells or humoral factors (antibodies) to foreign chemical and biological insults. The goal is to develop a better understanding of these responses for diagnostic purposes and in order to curb infections and neoplastic diseases.

Continued Use of Animals. Infectious diseases continue to cause death of large numbers of animals. A number of immune-deficient or hypersensitivity syndromes have been recognized and characterized in horses, cattle and dogs. Some cases, such as the severe combined immune deficiency syndrome in Arabian horses and the T-lymphocyte deficiency in Black Pied Danish cattle, are unique to these species (Perryman, 1979, 1982). These animals serve as important models to gain a better understanding of the complex interactions involved in basic immune responses. Simian acquired immune deficiency in monkeys has proven to be the best model for studying human acquired immune deficiency syndrome (Marx, 1984). Continued use of animals is needed for some other aspects of immune responses which are species specific. Bluetongue virus infection in cattle results in an IgE mediated immediate hypersensitivity while equine infectious anemia virus and visna virus of sheep have unique cytotoxic properties leading to immunopathology (McGuire, 1979; Stott, 1982).

Continued but Modified Use of Whole Animals. The technology evolving at present is providing the basis for reducing the need for as many animals as has been the case in the past. The use of laporoscopes for obtaining biopsies from lymph nodes and multiple blood samples obtained through catheters are providing lymphoid cells and macrophages which can be used in *in vitro* systems. These techniques will reduce the need for more surgery or euthanatizing animals in order to collect tissue for study. Pain associated with collecting blood samples is minimal. Laproscopy causes minimal pain since local anesthesia is used to obtain samples from fetuses or internal organs.

In most immunologic studies carried out on animals, the pain associated with studying the immune response is minimal, consisting of vaccinations and analyzing blood samples for evidence of immune responsiveness. In studies that require collection of tissue specimens, local or generalized anesthesia is practiced during the collection of specimens. These procedures may be carried out when tissues are collected during the study of immunologic diseases such as immune mediated glomerulonephritis in dogs, mastitis in cattle, or allergic pneumonitis in horses (Bennett, 1977; Hurvitz, 1982; Mansmann, 1975). Animals that have advanced signs of illness are euthanatized.

Use of lower mammals is practiced when possible. Often the initial immunological studies are carried out in rodents before the procedures are used on domestic animals. Many of the immune modulators such as interleukin 2 or interferon were tested in rodents before the products were tested in cattle, cats and horses.

Lower vertebrates and invertebrates are seldom used as substitutes for domestic animals in immunological studies. In some cases veterinary researchers use these animals as their principal research animal because they are studying disease processes in the respective species. Immune studies are carried out by veterinary researchers on fish as they develop fish vaccines (Roberts, 1978).

Use of Animal Components. An ever increasing amount of immunological research is being carried out on animal tissues. Concerted efforts are being made to collect lymphoid organs by biopsy, from abattoir specimens or at necropsy for immunological studies. When possible, the cells are made into cell lines (Theilen, 1982). These organs and tissues are cultured in vitro in an attempt to better understand the basis of immune responses and/or immunologically mediated diseases (Sawyer, 1973; Schultz, 1981).

The monoclonal antibodies are now being cultured in vitro. Rodents may be used to produce large quantities of these monoclonal antibodies in peritoneal cavaties; however, a considerable amount of cell selection is carried out in cell cultures. Methods are being investigated that will allow these cells to be cultured *in vitro*. Similar techniques are now being used to produce long term cultures of T-lymphocytes obtained from blood or tissue biopsies.

Use of Non-living Systems. At this time, non-living systems are not a significant part of immunological research in veterinary medicine. As the amino acid sequences of antigens and possibly antibodies are determined, it will be possible to synthesize these products *in vitro*.

Use of Computer Programs. Computers are used primarily to assist in analyzing data. Simulated models have not been developed to any extent for immunology in domestic animals. Before this is done, more basic information about the immune system will be required.

Microbiology

Statement: Microbiology in the context of veterinary research encompasses the characterization of structural, functional and pathogenic properties of bacteria, fungi and mycoplasmas of importance to the health and well being of animals and man.

Continued Use of Animals. Although a number of lower vertebrates have been identified that can be used as models for infectious disease research in domestic animals, there remains a number of infectious diseases with pathogenic properties that can be evaluated only in their domestic host species. Examples of these include the definition of enteropathogenic strains of *Escherichia coli* in pigs and calves which only attaches to intestinal lining cells of these species (Falkow, 1981; Isaacson, 1978); pathogenetic factors associated with pasteurellosis or the shipping fever complex occurs only in cattle because of lung morphology and other poorly defined factors (Slocombe, 1984); Johne's disease of ruminants of which the *Mycobacterium paratuberculosis* replicates only in these species; and epididymitis of rams and dogs associated with brucellosis (Gillespie, 1981). Animals need to be used to identify and understand the factors such as toxins that cause disease and to test protective measures that will curb disease.

Continued but Modified Use of Animals. Over the years, identification of many factors associated with causing some diseases in animals have been isolated and characterized. As the methods for identifying these factors are more commonly used, the factors can be identified by _in vitro_ methods with other microorganisms. These approaches have lead to better planned research protocols calling for fewer numbers of animals in experiments. For example, the recognition that "adherence factors" or pili antigens on _E. coli_ were closely associated with neonatal diarrheas in calves and pigs eventually led to the development of vaccines and monoclonal antibodies directed to these factors (Gillespie, 1981). The result was the development of these highly specific products to prevent these diseases thereby reducing the number of experimental animals used in searches for prevention and therapy of these diseases.

Where possible, lower vertebrates are being used in place of domestic species for research. For instance, studies on the effects of bacterial toxins such as those associated with clostridial infections are carried out in mice rather than sheep, horses or pigs. These toxins have a wide range of biologic activity in different species and this permits the use of laboratory animals.

Microorganisms are now being used with increasing frequency to study the genetic basis of virulence (i.e. disease causing) factors in microbiology. Genetic engineering (or recombinant DNA techniques) have provided researchers with this powerful tool to study antibiotic resistant microorganisms and adherence factors on pili.

Use of Animal Components. Animal components are being used with increasing frequency in microbiological research in veterinary medicine. Much of this has come about as researchers have focused on those properties of microorganisms that cause disease. Organ and cell cultures have been used to study attachment or adherence of bacteria to cells. These systems provided the basic clues for developing vaccines and monoclonal antibodies to prevent attachment or adherence of bacteria to cells with subsequent disease.

Use of Non-living Systems. Non-living systems are being used to synthesize products of microbes in a few instances. The newly acquired technology of protein synthesis of microbial antigens will provide important technology for making purified products for vaccines. It will also be possible to make toxins and/or toxoids, portions of pili and cell membranes.

Use of Computer Programs. Computer programs are used to define basic genetic and protein sequences. This information is then used to synthesize antigens for vaccines.

The application of the computer technology to replace animals in microbiological research remains poorly developed at this time. Suitable disease models have not been proven, primarily because of the lack of understanding of the highly complex host and microorganism. It is still too early to determine the effectiveness of these programs as a replacement of animals in microbiological research.

Molecular Biology

Statement: Molecular biology is the science which characterizes the functional and structural aspects of basic processes of life. Current research includes manipulation of cellular genetics, synthesis of biologically-active proteins and cell membranes.

Continued Use of Animals. Presently the studies are confined almost exclusively to *in vitro* systems. Eventually the products of these studies will be inoculated or injected into animals in order to evaluate their value. A few experiments have been carried out evaluating the effectiveness of a recombinant DNA vaccine for foot and mouth disease in cattle (Bachrach, 1982; Boothroyd, 1981; Kleid, 1981). Similar studies are being carried out with the growth hormone made by recombinant DNA (Doehmer, 1982). Application of these products to animals will increase in the next decade. Increased animal research will be needed in order to determine if vaccines, hormones or inserted genetic materials made by these means will be safe and efficacious. It is important to determine if the products manufactured *in vitro* have effective biological functions.

Continued but Modified Use of Whole Animals. In nearly all instances, initial studies of molecular biology will be carried out in cell cultures and lower mammals before being conducted in domestic animals.

Use of Animal Components. Presently most of the basic and applied research in molecular biology is carried out in cell or microbial culture systems. Recombinant DNA technology is carried out in bacteria, yeast and mammalian cell culture systems.

Use of Non-living Systems. Non-living systems are being used in molecular biology studies. Artificial cell membranes are being synthesized to study basic processes, and protein hormones can be synthesized in non-living systems. Protein synthesizers are being used to make hormones and antigens (Beale, 1982; Bittle, 1982). As researchers characterize the important active chemical configurations of biological molecules, there will be greater use of these systems.

Use of Computer Programs. The use of computers and computer programs is a necessity in molecular biology. The finite detail and the need to characterize chemical sequences in the genetic code or in defining biologically active chemical structures in proteins and cell membranes requires computer programs (Bittle, 1982; Staden, 1977; Staden, 1980). As the basic chemical structures are defined, models are being developed which can be used in future research.

Nutrition and Metabolic Disease

Statement: Nutrition is the study of the properties, intake, digestion, assimilation, and utilization of food. Nutrition includes the study of nutrient requirements, deficiencies, and toxicities. Metabolic diseases are those diseases in which aberrations of metabolism are the predominant feature. Metabolic disease is broadly interpreted to include many diseases in which the primary causes are of hormonal, nutritional or genetic origin. There is much overlap between biochemical research and research on nutrition and metabolic disease.

Continued Use of Higher Mammals. Much applied research on the nutritional needs of animals must be performed on the species of interest because of interspecies diversity of nutrient requirements and tolerances. We simply do not know enough to predict the nutrient requirements and tolerances of animals without *in vivo* feeding experiments. Closely related species can vary considerably in their nutritional needs and tolerances. For example, a daily intake of the trace mineral copper that is well tolerated by calves can be quite toxic to sheep (Ammerman, 1980). Rats do not require vitamin C in their diet, whereas guinea pigs do (Bell, 1978).

In the case of inherited metabolic diseases, the number of species available for research are often limited because the disease may only be known to occur in a few species. For example, a disorder of bile pigment metabolism known as Gilbert's syndrome is known to occur in only two animal species, the squirrel monkey and the sheep. This syndrome could not possibly be investigated in rats since no rats have yet been discovered that have the syndrome (Cornelius, 1970; Portman, 1984). The disease of cattle called bloat in which the animals are unable to expell gas generated during food fermentation in their stomachs cannot be studied in mice or rats because these two species have no fermentation in their stomach (Church, 1976).

While there are many lower mammal models for some animal diseases, such as diabetes (Festing, 1979; Desnick, 1982), these models may not be suitable for research on domestic animal disease for a variety of reasons. A disease in rodents may differ in significant details (age of onset, for example) from a similar disease in dogs. The interaction of a metabolic disease with diet may be difficult to study using an omnivorous rodent as a model for a carnivorous cat. Finally, there is the ethical question of why one animal species should be used to solve the disease problems of another. If an investigator is interested in diabetes in dogs, why should he use rats to do research on a dog disease, especially when the results from research with the rats may turn out to have limited applicability to dogs?

Much of the nutritional research done with domestic animals is done for the benefit of these animals rather than as a model for man or some other species. The results allow us to feed our animals in the best possible manner.

Continued but Modified Use of Whole Animals.

Reduction of insult. In many feeding experiments, the presence of an "insult" is debatable. Often the diets to be tested are all considered "normal" in that all of them fall within established guidelines for minimal content of essential nutrients, and the goal of the experiment is to measure palatability, efficiency of weight gain, or rate of growth. In the case of determination of minimum requirements or tolerance of nutrients, it is necessary to keep reducing the dietary content of the nutrient under study or keep increasing it until the appearance of deficiency signs or toxicity, respectively.

In the case of many inherited diseases, the insult is brought about by the animal's own genes. The only way to prevent the insult would be extinction of the strain.

More extensive use of blood-sampling catheters would make repeated sampling with needles unnecessary. General anesthetics are not feasible with long-term experiments and may interfere with many short-term experiments.

Substitution of lower mammals. In applied nutrition, substitution is usually impossible. As pointed out above, evenly closely related species can have quite different nutritional requirements and tolerances. Rats cannot be used to determine the trace mineral requirements of dogs.

Substitution of lower vertebrates. Except for the most basic of studies, such as those on the cellular uptake of nutrients or the cellular toxicity of nutrients, lower vertebrates cannot substitute for higher mammals, particularly in nutritional studies because of the great interspecies diversity of nutrient requirements and tolerances.

Use of Animal Components. Animal components are quite useful in the exploration of the most basic aspects of nutrition and, especially, metabolic disease. The isolation and characterization of abnormal proteins, nucleic acids, fats, and carbohydrates from animals with metabolic diseases is essential to further understanding of these disorders. Likewise, cell cultures can yield considerable amounts of basic molecular and cellular information. Whole animal research is still needed to understand and integrate basic findings and also as a source of body components to study.

The usefulness of animal components in nutritional research is limited in that the handling of most nutrients at the cellular and subcellular level is considered to be biochemistry rather nutrition. However, cell cultures and organ perfusion are useful in the study of trace minerals and artificial nutrients like sweetness.

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Classes of alternatives. Tissue cultures are quite useful in exploring the basic aspects of metabolic diseases. Cultures of cells from individuals with inherited metabolic abnormalities are quite useful in exploring the biochemical and cellular basis of metabolic diseases, and there is potential for increasing the use of cell cultures in place of whole animals. For nutritional research, the usefulness of cell cultures is limited.

Invertebrates, microorganisms and plant classes of alternatives are of little direct use in nutritional and metabolic disease research.

Use of Non-living Systems. Physical systems are of some use in nutritional and metabolic research. *In vitro* reactions of synthetic abnormal body molecules have some usefulness in the study of metabolic disease. These studies do not substitute for any whole animal research.

Use of Computer Programs. Computer programs are used to study the flow of metabolites through biochemical pathways when the properties of some of the enzymes in the pathway are altered as in some metabolic diseases. Computer programs are also used to optimize diets for animals and to simulate the projected growth rate and feed efficiency of animals on various diets.

The use of computer programs, especially to simulate the kinetics of metabolic pathways, has great potential. The more significant results of such studies must be verified in animals because no programs yet exist that can simulate the total molecular environment of body cells.

Parasitology

Statement: Parasitology is the study of the relationship between organisms where one organism, the parasite, lives on or in the other, the host, and receives nutrients from the host. This study includes the natural history of this relationship, the deleterious effects on the host, the effects of population dynamics and environmental host factors and the results of medical intervention upon this relationship (Flynn, 1973).

Continued Use of Animals. Because of the complex interactions between the host and parasite and the effects of host environmental and social factors, the continued use of whole animals is essential to the understanding of this relationship. For example, in most cases of parasitism, a balance is maintained between host and parasite. Altered host factors which may be the result of conditions of domestic production can produce advantages for the parasite which will damage the host. Examples of the factors mentioned above and how changes in these factors can alter the balance between host and parasite are as follows: Host environmental factors include such items as the type of pasture animals are kept on, the extent of irrigation or ground moisture, the ambient temperature, the elevation and the season. These directly affect the health of the animals and the survival of the parasite if the parasite ova are shed in the feces. Social factors include the density of animals and the amount and extent of social interaction. Increased density favors the transfer of parasites, for example. In addition, most species of animals establish social hierarchies with lower ranked animals being placed under greater social stress. Such stress carries increased risk of susceptibility to disease. In many instances, parasites are host specific and therefore can only be studied in their natural host.

Continued but Modified Use of Whole Animals. The understanding of the basic biology of the parasite has led to rapid advances in parasitology. For example, studies on immune receptors, membrane transport and the basic biochemistry of the parasite has indirectly led to a reduction in the numbers of animals required on studies. In addition, knowledge of the parasite has allowed for more refined or more sharply focused studies in whole animals. Breakthroughs in immunology may allow for immunizations of the host against the parasite (Soulsby, 1979).

Pain produced by reproducing naturally-occurring disease is alleviated when feasible, providing such alleviation does not interfere with the mechanism being studied.

Substitution of lower mammals is commonly practiced when possible. However, parasites are frequently host-adapted and may not produce the same pattern of disease in another species.

Lower vertebrates and non-vertebrates are commonly used. The *in vitro* study of the parasite itself has provided fundamental knowledge leading to a better understanding of the process of parasitism and the mechanism of elimination or control.

Use of Animal Components. Cellular systems are being used and are providing breakthroughs in the understanding of parasitism. For example, white blood cells from mammalian species are being cultured for and infected with protozoan parasites such as Leishmania to understand the mechanisms of host-parasite interaction.

Use of Non-living Systems. Parasites are grown in fluid cultures to gain insight into the biology of the parasite. For example, *Histomonas meleagridis*, the cause of Blackhead in turkeys, can be grown in artificial media. Such studies rapidly advance knowledge with the end result of reducing the total number of higher animals required for studies.

Use of Computer Programs. Computer use will aid in the analysis of studies on the parasite and in the analysis of data from field studies on parasitism. Modeling can help in predictive planning and in reduction of the total numbers of animals required for study. For example, in African Trypanosomiasis, computer manipulation involving parameters of reservoir populations, vector populations, climatic changes, etc., may provide insight on transmission to livestock/humans and identify areas for host effective control strategies and experimentations.

Pathology

Statement: Pathology is the study of the nature and causes of diseases and of the structural and functional consequences of these diseases on the affected organism. It encompasses both morphological and clinical pathology. Emphasis is on abnormal structural and functional changes caused by a large variety of injurious agents. Integration of changes at the molecular and cellular level with influence on organ systems and ultimately, the whole animal, is an essential feature of the discipline.

Continued Use of Animals. Clinical manifestation of disease is the result of complex interactions of abnormal structural and functional (pathological) changes at molecular, cellular and organ system levels requiring the use of whole animals. Analysis of blood, serum and tissues is the principle means of assessing pathologic changes. Basic mechanisms of inflammation can only be studied in living systems because the responses center on blood vessels which respond to neurological stimuli, vasoactive amines released from basophils, mast cells, platelets, and serum factors such as kallikrein, clotting components as well as cells such as neutrophils and monocytes (Slauson, 1982; Thompson, 1984). An understanding of the linkage between disturbances in major organ systems of the body requires the whole animal. Systemic lupus erythematosus which occurs in dogs, cats and possibly horses affects the immune, hematopoietic, renal, synovial, integumentry and sometimes the cardiovascular systems (Halliwell, 1978, 1982). As a consequence, it is not possible to study and understand the disease process unless the whole animal is studied.

Continued but Modified Use of Whole Animals. Some reduction in the use of whole animals might be possible if biochemical techniques and molecular biology enable more sharply focused studies. By using biochemical and hematological tests on peripheral blood, it is possible to gain a better appreciation of pathophysiologic changes occurring in animals. During infectious disease processes, the white blood cell count (neutrophil) are elevated (Schalm, 1975). Serum samples collected from animals with liver disease contain enzymes released from damaged cells into the serum (Kaneko, 1980.

Experimental reproduction of disease may lead to pain; however, attempts to alleviate pain or euthanazia are used to minimize pain. In some instances, in order to determine the pathogenesis of disease such as mastitis in cattle the rapid swelling will lead to some pain. Understanding the cause of swelling and pain such as that caused by prostaglandinins now make it possible to develop treatment regimes (Emau, 1984; Giri, 1984.)

Substitution of lower mammals (e.g., laboratory rodents) is already being practiced where appropriate. As suitable correlations with lower mammals are made, these species are used in increasing numbers. Since there are important interspecies differences in structure/function from molecular through whole-animal level of organization (Tyler, 1983), study (or at least validation) is needed in the animal species of concern.

Lower vertebrates and non-vertebrates are seldom used as substitutes for pathology of higher animals. These species are used primarily for basic or comparative studies within the species (Roberts, 1979). Much information remains to be learned about basic disease and host responses in these species.

Use of Animal Components. Many aspects of molecular and cellular biology are being investigated in cell, tissue and organ culture. These systems are where much of the information on pathogenetic mechanisms at the cellular and subcellular levels are being obtained. The mechanisms so derived, however, must be explored in the intact animal because of the critical involvement of integrative control systems in the whole animal (immune, neural, endocrine, paracrine), and the influence of one organ system upon another. Toxins from microbial agents potentially implicated in causing pneumonia can be isolated, studied in culture systems, and toxins evaluated in cell culture systems. The extent to which they are actually capable of causing pneumonia, and the circumstances under which they do so can only be investigated properly in intact animals (Gillespie, 1981).

Use of Non-living Systems. Non-living systems are not used in morphologic pathology because the nature of the discipline is to subjectively describe changes in tissues. On the other hand, clinical pathology often uses non-living systems to analyze biochemical changes in body fluids (Kaneko, 1980). Initial studies to develop assays to identify specific chemical substances such as bilirubin, liver enzymes, etc., can be performed by chemical analyses. Eventually the tests must be carried out on animals with natural or experimental disease.

Use of Computer Programs. Computers have not been used to any significant extent in pathology. Morphologic pathology is a subjective discipline depending upon visual interpretative skills. Clinical pathology has a greater possibility of incorporating computer programs into future studies. Simulated models may be used to predict biochemical or hematological changes.

Pharmacology

Statement: This discipline is the study of drug action in the body. It is concerned with the absorption, distribution, action on cells, metabolism, and elimination of drugs.

Continued Use of Higher Mammals. Much veterinary pharmacological research involves testing of new drugs for efficacy, toxicity, metabolism, dosage forms, and routes and rate of elimination. While preliminary studies might be performed in vitro or in lower animals, if such studies are promising, the drug will be tested in the animal species for which it was being developed (i.e. the target species). The question to be answered is whether the first exposure to the drug by individuals of the target species should be under controlled conditions in individuals belonging to an institution or under less well controlled conditions by the pets and farm animals of the public. Scientific, ethical, and political considerations point toward a rather obvious choice.

The continued use of higher mammals is essential because of the diversity of drug action and drug needs of various species (Albert, 1979). For example, cattle suffer from a common disease called bloat in which gas generated during fermentation in their forestomach (in the rumen) is too foamy to be expelled. As a consequence, the gas accumulates until the pressure on the diaphragm from the expanding rumen makes breathing insufficient for survival. There are drugs designed specifically to treat this condition but more effective drugs are needed. One class of drugs used to treat bloat is antifoaming agents. Interestingly, antifoaming agents can be initially tested in vitro by assessing the rapidity with which they can disperse protein and pectin foams. Eventually, promising antibloat remedies must be tested in live animals that have the disease (Church, 1976). It would be impossible to test bloat remedies using any species but cattle or related ruminants, like sheep or goats, which ferment food in their stomachs.

The similarities among closely related species of drug action and metabolism allows some interspecies extrapolation. For example, primate species are relatively good pharmacologic models for each other and the same is true within rodent species. However, rodents are not nearly as good models for primates (including man) as the primates are for each other (Caldwell, 1981).

An example of diversity among species of drug potency occurs with xylazine which is a tranquilizer and sedative. Xylazine is 20 times more potent for cattle and sheep than for horses, dogs and cats, and there is no explanation for this difference at present. A safe dose for a horse is fatal for a cow (Rossoff, 1974).

Perhaps the most extreme example of species diversity occurs with norbormide. This compound is fatal to rats in tiny doses but is relatively nontoxic to nearly all other species including such closely related species as mice and rabbits (Albert, 1979; Roszkowski, 1965). The possibility always exists that any new therapeutically promising drugs could be a "norbormide" for our domestic animals (or man). Unless new drugs are tested in animals that are close models or the same species as the target species for which they are to be marketed, the probability of a pharmacological disaster, like that of thalidomide, is increased. Until we understand drug receptor sites and actions better in various species, there can be no substitute for testing drugs in each species for which they are to be marketed.

Some studies in pharmacology could probably be performed with lower drug doses than are used at present. Such studies are those involving determination of drug distribution and structure of metabolites, although in some cases, the results of such studies are dosage dependent, and there could be no substitute for higher doses. Additionally, the sensitivities of the methods for drug assay may dictate the minimal dose.

Studies on drug toxicity fall into the disciplines of both pharmacology and toxicology. There can be no substitute for performing studies in higher mammals before drugs are released for sale. Since veterinary drugs that pass preliminary screening will be used in animals, it is better that toxicity be evaluated in institutions rather than at the expense of animals belonging to individuals. Because of the diversity of species response to drugs, pointed out above, eventually toxicity must be evaluated in the target species. With the exceptions of euthanasia agents, studies can be designed to ascertain the minimally toxic dose without causing severe toxic signs or death.

Substitution of lower mammals. The use of lower mammals is an important part of pharmacological research and is beneficial for large-scale screening of new compounds for pharmacological effects and for drug toxicity testing. These animals are very useful for studies on the molecular basis of drug action. The limitations in using lower mammals as substitutes for higher mammals were stated above.

Lower and non-vertebrates are of some use as substitutes for mammals in pharmacological research. Basic cellular studies can use invertebrates. Examples are the use of squid axons for basic neuropharmacological research and barnacle muscle fibers for basic myopharmacological research. In these two examples, the large size of the invertebrate cells makes them useful substitutes for their mammalian counterparts. Invertebrates and lower vertebrates are also sources of pharmacological agents, but do not really substitute for mammals in this regard.

Use of Animal Components. Culture systems, using mammalian cells, are of great use for large-scale examination of toxic effects and for studies on the molecular basis of drug action.

Cultured cells cannot substitute for whole animals in many types of research because some body cells cannot be cultured successfully at present, and some drugs are intrinsically toxic to cell cultures (i.e., many drug preparations for use on the skin will kill cultured cells at concentrations too low to be useful). There is, however, reason to hope that eventually skin and eye irritation tests will be performed with tissue or organ cultures. The recent successful culturing of human skin for grafting is an example of progress toward a goal of having dependable substitutes for whole animals (Gallico, 1984). Such in vitro substitutes still are not close enough to their in vivo counterparts to be reliable at present.

Invertebrates, microorganisms, and plants are of great use in pharmacological research as sources of active compounds, but this use does not really substitute for whole animals.

Use of Non-living Systems. Non-living systems have some use in pharmacological research, such as in the use of artificial membranes to study drug transport into cells and in the study the chemical reactions of drugs with each other and with body substances. It is doubtful that this approach is a useful adjunct to whole animal experiments.

Use of Computer Programs. Computers are already used extensively in that branch of pharmacology known as pharmacokinetics which studies the distribution and rate of elimination of drugs. It is possible to predict rather accurately how a drug will be handled when given at various doses by various routes once the parameters have been determined for just a few dosages and routes. However, it is still necessary to do the first few studies in animals. Also, there are instances when the mathematical models have not proved accurate. The models reach their limits of prediction at high drug doses when cell uptake and metabolic routes become saturated, when toxic signs occur, or when diet or disease alters tissue function and drug uptake (Baggot, 1983; Gibaldi, 1982).

Physiology

Statement: Physiology is the science which pertains to the functions of the living organism and its parts. Physiology is often subdivided according to the systems of the body: digestive, cardiovascular, respiratory, nervous, endocrine, urinary, reproductive, and muscular and skeletal systems. Physiology is also studied as the physiology of individual organs, i.e., heart, brain, kidneys, etc., or individual cells, i.e., cellular physiology. Pathophysiology is the study of function altered by disease.

Continued Use of Higher Mammals. Before pathophysiology can be studied, the normal physiological state must be known. Because many of the domestic species have not been used as human models of disease, little is known about their physiology or pathophysiology because of lack of funding for research on animals.

The varied species-dependent physiological responses to stress and/or disease make continued use of specific species of animals necessary. An example of a species-dependent physiological response to stress is exertional rhabomyolysis (azoturia, tying up or Monday morning disease) of horses (Lindholm, 1974). An example of a species-dependent physiological response to disease is traumatic reticulo-pericarditis in ruminants (Blood, 1979). Similarly, species-specific diseases alter the physiology of one animal of a particular species quite differently than a similar disease might alter the physiology of another animal of a different species.

Physiological research on domestic animals cannot, by the nature of the research, appreciably disturb the physiology of the animal from the normal. This fact has led to striking improvements in techniques used, for example, by using fiberoptics and telemonitoring of physiological processes, the animal experiences little or no pain or discomfort during these studies. Pain, stress or anxiety greatly alter normal physiology.

Continued but Possible Modified Use of Whole Animals. Because so little is known about most physiological processes in domestic animals, little reduction in numbers appears likely. Reduced numbers of animals have come about because of the considerable expense involved in the purchase, care and feeding of these large animals. Most veterinary physiologists are trained in statistics and keep their animal numbers to a minimum to avoid increasing the already high costs of doing research on animals for the sake of animals.

As referred to above, the nature of physiological research dictates that the natural (normal) status of the animal be maintained. Pain or discomfort from extensive surgery or invasive procedures runs counter to normal function. Similarly, in pathophysiological studies, any procedure not a part of the pathological process may lead to erroneous results. Because most scientists do not want to publish erroneous results, there are strong scientific incentives to keep insult at minimum.

Lower species have been and still are extensively used in physiology, especially cellular physiology and neurophysiology, to elucidate fundamental processes. These studies on lower animals are of limited value in systemic physiology because of uniquely different physiological mechanisms operating in these diverse species as compared to the larger domestic animals. Many studies are presently using non-vertebrates, especially those involving cellular physiology and neurophysiology. This is particularly true concerning cell membrane function. Again, these studies are of limited value because of differences among species.

Use of Animal Components. Animal components have been used for more than 40 years. Early studies on the excised mammary gland using extracorpular circulation and/or organ perfusion produced classic results. More recently, other isolated organs have been studied in isolation (kidney, embryo heart, liver, spleen, uteri, gut, skeletal muscle).

Much cellular physiology has been studied *in vitro*, i.e., squid axon, tissue cultures of nervous tissue, renal tissue, cardiac cells, etc. These studies give insights into biochemical and biophysical phenomena, but it is not possible to develop vascular, neural, and endocrine controls in tissue culture that will function similarly to the way control is exercised in an organ in an intact animal. Blood vessels and nerves will not grow in tissue culture (Schubert, 1984).

Use of Non-living Systems. Physical systems, such as artificial hearts, lungs, kidney-dialysis machines, have been, and are being developed to mimic natural organs. Their use in pathophysiology is limited because it is difficult to mimic disease in these non-living systems.

Use of Computer Programs. Computer models have been developed which mimic simple neural networks, such as those of the hippocampus (Horowitz, 1975) and simple sensory systems (Salu, 1983). Other models have been developed to simulate the cardiac conduction system (Malik, 1983), the pulsatile flow in arteries (Jelinek, 1979), the respiratory system of birds (Hempleman, 1979), and the thermoregulatory system of rats exposed concurrently to cold and to acceleration fields (Giacchino, 1979). These computer models require data derived from in vivo studies. Any data derived from computer models must be confirmed by living animal studies.

On-line computers are being extensively used to correlate large quantities of simultaneously gathered data and revealing interelationships among various physiological and pathological events.

Radiology/Radiation Biology

Statement: The radiological sciences are concerned with the evaluation, diagnosis, and therapy of living systems, as well as analysis of the effects of radiation on biological systems, particularly as they relate to neoplastic processes. Because these disciplines pursue clinically oriented research, they usually involve the use of living animals or animal patients with specific disorders (Ackerman, Navicular...1977; Ackerman, Radiographic...1977; Millman, 1979; Suter, Oropharyngeal...1980; Suter, Portal...1975; Turrel, Radioactive...1984; Turrel, Radiotherapy...1984).

The diagnostic portions of radiological sciences include: (1) conventional radiography, where x-rays are passed through the body to evaluate structure and function of various organ systems; (2) angiography, where catheters are passed into various blood vessels within the body to evaluate the structure/function of specific organs; (3) computed tomography, where x-rays are used to create cross-sectional representations of the body's anatomy for morphologic evaluations; (4) nuclear medicine, where various radioisotopes are introduced into the body and their collection within various tissues in the body is analyzed to describe organ structure and function; 5) ultrasound, where a high-frequency sound beam is passed through the body to create pictures representing the anatomy of the organs being observed; and (6) radiation therapy, where the harmful effects of various types of radiation are employed to destroy cancers in animal patients.

Continued Use of Whole Animals. Because the veterinary radiological sciences are heavily involved with animal patient care, whole animals are employed almost exclusively for research in these disciplines (Kasstrom, 1972; O'Brien, X-Irradiation... 1971). Furthermore, because each species has different anatomical and functional relationships among its organ systems, a new diagnostic technique in one species cannot be applied automatically to another species without subsequent validation (Morgan, 1977). Any investigations which attempt to quantitate organ function or evaluate response to various treatment regimens, obviously require the use of live animals. Many of the anatomical investigations even require the use of live animals since the relationships and size of organs may change dramatically after death (Amis, 1984; Hornof, The Use...1979; Munger, 1978; Silverman, Alterations...1974; Silverman, Influence...1975; Silverman, Radiographic...1974; Suter, Swallowing...1978). Examples would include studies involving the lungs which collapse and become congested with fluid after death and the kidney which shrinks dramatically in size when blood pressure is lost.

Many investigative techniques also require surgical manipulation of normal experimental subjects (McGahan, 1983;

Nyland, Sonographic...1982; Nyland, Ultrasonic...1983; Suter, Oropharyngeal...1980). A good example would be an attempt to develop a completely noninvasive technique for quantitating individual kidney function in patients. Currently, the only method available for quantitating individual kidney function is to surgically enter the abdomen and catheterize the ureter coming from each kidney. In this way, if a known amount of substance which is excreted by the kidney is injected, the amount excreted by each kidney can be measured and kidney function evaluated. Obtaining information about the function of individual kidneys is clinically important since a non-functioning kidney may be a focus for infection that cannot be cured medically and needs to be surgically removed.

Unfortunately, an invasive test as described would not be practical because of the danger to the patient and the possibility of postsurgical complications such as ureteral leakage. If a noninvasive diagnostic test could be developed, this may have far-reaching implications in aiding and expediting recovery of animal patients with kidney disease. If a new technique for assessing individual kidney function is proposed, such as observing the rate of accumulation of a minute amount of radioactive tracer in the kidney, it must be validated. This requires that a number of experimental animals be subjected to anesthesia and catheterization of their ureters. Furthermore, since experimental animals are in good health, and the technique will eventually be used in patients with abnormal kidney function, the kidneys must be surgically manipuated and damaged to simulate a kidney disease. This means that these animals could not be allowed to recover from anesthesia and must be sacrificed at the end of the study. It would be the intent, however, in this type of study that the sacrifice of these few experimental animals would be far outweighed by benefits to animal patients saved by the use of this new technique.

Since the overall goal in the radiological sciences is to improve patient care, most research is aimed in this direction.

Continued but Modified Use of Whole Animals.

Reduction in numbers. Because of the biological variability of individual subjects, relatively large numbers of subjects are needed to provide statistically accurate results. All diagnostic tests are developed by examining both normal and abnormal animals. Even within completely normal animals, a wide range of values is encountered for any given diagnostic test. Generally, there is also considerable overlap in the values obtained in both normal and abnormal animals. It then becomes critically important to accurately define the limits of the range of normal values in a given test. The accuracy and confidence that one can have in these limits is related directly to the number of animals tested. In the example of the kidney study previously mentioned, the use of fewer animals might result in only being 50 percent sure that a kidney in an individual patient needs to be removed rather than being 95 percent sure.

Experiments which do not attempt to quantify function but merely investigate anatomical change also require the use of large numbers of live animals. In dead animals, the heart stops beating, the lungs collapse, and various organs shrink or expand in size preventing the use of measurements obtained in these animals from application to clinic patients (Silverman, Alterations...1974).

Reduction of insult. Generally, experiments are designed to minimize physical insult or anguish in the subjects. Since vast differences in organ structure and function between species exist, the use of lower vertebrates would not be feasible.

Use of Animal Components. Tissue components can and are often used for research in radiological sciences. These generally involve the use of bone tissues to evaluate structure/function and pathological relationships. These research activities will undoubtedly continue and often involve the use of specimens obtained from animal patients after natural death and consent of the owner for necropsy (Hornof, Osteochondritis...1981; Hornof, Radiographic...1980; O'Brien, Navicular...1975; O'Brien, Radiographic...1981).

Use of Non-living Systems. The design of physical systems to mimic various organs can be used to better understand organ function. A prime example of this kind of investigation would be evaluation of the heart using a physical pumping device. These devices have been used to evaluate various diagnostic techniques. They tend to be very simple models of a much more complex organ that perform similar functions. With such devices, the effects of specific alterations of the model can be determined. A simplistic example might be to increase the stroke volume of the pumping device and determine the changes in total output. The information gained from such an experiment could predict the response in the actual living system. Such devices can be employed to develop much more refined and sophisticated techniques to reduce the actual numbers of animals needed for statistically significant results.

Use of Computer Programs. Living biological systems are highly complex and the modeling of whole organ function is only just beginning. Because of the extreme complexity of biological systems, it has only been recently that the computer hardware required is becoming affordable. This hardware has permitted the development of these complex computer programs. Unfortunately, these programs require extensive validation in living biological systems with various interventions to verify accuracy.

The availability of powerful computers in the laboratory may actually result in an initial increase in the number of experimental animals employed in validating computer modeling techniques in the near future. As was the case with the development of new diagnostic techniques, computer models must in fact perform nearly identical to the living biological system. This means that if a complex organ such as the lung is to be modeled, the exact response of the lung to a given intervention must be known in the living system. This requires that large numbers of animals be subjected to extensive testing to determine the range of responses that would normally be anticipated to given interventions such as drugs or inhaled irritants. The development of tested computer models will eventually result in reduced need for live animal experiments in the future. If, for example, a new drug is discovered which causes a specific reaction such as constricting vessels, the effects of this drug on the lung can be acurately predicted by employing the computer model rather than by again using large numbers of experimental animals.

Surgery

Statement: Surgery is the correction of defects and diseases and the repair of injuries using the surgeon's hands and instruments. Surgery is particularly important to pet practice and equine practice, but it also comprises a small but important aspect of food animal practice.

The present use of animals by the discipline of veterinary surgery is aimed at developing and refining new surgical techniques, developing and testing new materials (such as the artificial hip joints now being implanted in some dogs), and teaching the state of the art in veterinary surgery to veterinary students.

The Federal Animal Welfare Act presently requires that the methods of anesthesia, analgesia, and post-operative care for animals involved in surgical studies be approved by the attending veterinarian at every research facility (U. S. Dept. of Agriculture, 1977).

Continued Use of Animals. For a large segment of surgical research, there is no effective substitute for use of whole animals. The reasons are that many phenomena in surgery involve motion, healing, tissue tolerance, and complex physiological and even electrical relationships that cannot be duplicated outside an intact animal. An orthopedic device, for example, must not only be strong enough to carry the load intended for it, but must allow healing, not be immunogenic, and not cause the lysis or weakening of adjacent bone. A common problem, and a topic of active research, is how to prolong the life of orthopedic implants such as steel plates and prosthetic joints (Brosnahan, 1981; Creed, 1971; Leighton, 1979; Radin, 1982; Ronningen, 1983). Presently, a significant percentage of such devices eventually causes weakening of bone near the point at which they are connected to the bone. This weakening can eventually cause a surgically repaired limb to re-fracture. Studies of ways to improve the performance of such devices require long-term post-operative studies in whole animals.

Studies which are concerned with techniques and not devices still frequently require whole animals. The mere performance of the technique in a dead animal or on a model of an animal would not answer questions about the long-term success of the technique. How well did the repaired organ function post-operatively? How long did the repair hold up? Was the correction permanent or only temporary? Finally, it is sometimes necessary to create the defect that one intends to correct. One cannot determine whether or not a method is a good method for repairing a fracture without actually creating the fracture.

Continued but Modified Use of Whole Animals.

Reduction in numbers. The numbers of animals in veterinary surgical research projects are already severely limited due to the cost of the animals and the paucity of funding for purely veterinary research. It is very doubtful that there are any research projects using a larger number of animals than the minimum necessary for statistically significant results.

One way to reduce the numbers of animals used in both research and teaching is to "recycle" animals from one project to the next. That is, after project A is completed, use the dogs from project A on project B, assuming that project B studies a part of the animal not manipulated in project A. Most veterinarians oppose the repeated use of experimental animals on humane grounds. It is generally considered abusive to subject an animal to two surgeries unless such use is a necessary part of a single experiment. There is also the possibility that drugs given or procedures performed during the first study might alter the results of the second study in unforseen ways. Repeated uses of the same animal should be minimized.

Reduction in insult. The insult to animals in surgical experiments and classes may be reduced in several ways:

If the animals are not allowed to regain consciousness following a surgery, then they cannot experience any post-operative discomfort. If a research project can be sucessfully performed as a "terminal" study, i.e., the subject animal is euthanized while still under anesthesia, then the stress to the animal involved is much less. The sacrifice made is that healing and post-operative function cannot be observed afterward. For many studies, this would defeat the purpose of the experiment, but for others it is a satisfactory way to insure that the animal does not experience any pain or discomfort.

Substitution of lower mammals. Unlike situations where animals are used as models for man in human surgery, the animal subjects of veterinary surgical research are usually the species for which the technique is intended. The substitution of one species for another would not usually achieve any particular humane goal. Substituting a pig for a dog, for example, would not necessarily be preferable since pigs are as intelligent and as advanced phylogenetically as dogs are.

Rodents and rabbits can sometimes be substituted for other mammals. The femoral vessels of the rat, for example, can be used to teach and practice microsurgery techniques. The vessels of the rat would be used in this case as a model of smaller vessels in larger animals. Both rabbits and rats have been used in basic science studies pertaining to surgery, such as studies about the long-term fate of suture materials, the significance of wound infection, and the role of the greater omentum in the healing of the gut after intestinal resection (Macnamara, 1984; Marchant, 1984).

In a great many other studies, the small size of rabbits and rodents makes them unusable for studies pertaining to larger animals. There would be no way, as an example, to scale the forces involved in a hip joint replacement for dogs down to a meaningful study in the rat. Many of the internal organs and bones of rodents and rabbits are simply too small to be worked with except in studies of microsurgery.

The substitution of lower vertebrates and invertebrates for higher mammals is simply not possible. The anatomy of these latter creatures is too different from that of mammals to make any surgical studies relevant.

Use of Animal Components. Animal components receive considerable use in well designed surgical studies. A thorough knowledge of anatomy is the most important foundation of surgery. Cadavers are used almost to the exclusion of live animals in the study of anatomy.

Freshly dead animals, obtained from pounds, are also used in mechanical studies of the stresses and strains to which normal, living tissues are subjected. For example, freshly dead dogs can be used to study the stresses and forces that lead to intervertebral disc problems and dislocations of various joints in the dog (Ashman, 1982; Vasseur, 1982). Freshly dead horses have been used to study the mechanics of running and leg injuries in horses. Animal parts obtained from slaughterhouses and meat markets are also used in research (Riggins, 1983).

Use of Non-living Systems. Non-living systems, such as mechanical models of living systems, have a few very limited applications. Some purely mechanical systems may be used to judge the corrosion resistance of surgical implants (Cahoon, 1981). Other purely mechanical systems can be used to test orthopedic implant devices, or artificial heart valves, and other types of implantable devices for their resistance to wear and fatigue under conditions similar to those they would encounter in actual use (Rousseau, 1984; Zand, 1983). While such studies are useful, their weakness is that they are one-sided. They consider only the device's performance and not the animal's response to the device.

Use of Computer Programs. Computers are useful in the compilation of data from other experiments. They are used extensively to process data from patient records (to evaluate which treatment gave the best results in treating a given condition). They can also process data from pathology reports to obtain such diverse information as the long-term survival from different types of tumors, the effects of spaying a dog at various ages on the dog's subsequent incidence of breast tumors, the likelihood of a racehorse breaking a leg on the track after receiving a particular medication, etc.

The ability of the computer to make prospective predictions as to the potential efficacy of new surgical techniques, or to predict the reaction of an animal to a new device or technique is limited. There have, at this time, been few, if any, attempts to develop such software for veterinary applications. Mathematical modeling techniques have been used in some studies with human surgical applications (Ghista, 1983; Ishai, 1983). Development of mathematical models for veterinary surgical applications should be explored, but this author is not aware of computer software with veterinary surgical applications that is available at this time.

Toxicology

Statement: Toxicology is the study of the effects of chemicals on biologic systems with emphasis on the mechanisms of harmful effects and the conditions under which harmful effects occur. In the veterinary arena, toxicologists are concerned with intoxications of drugs or other chemicals and the demonstration of their safety or hazard prior to their entry on the market.

Continued Use of Animals. There are certain times and situations in which there is a clear need to use whole animals in toxicological research. Certain forms of plant poisons cause diseases in some species while in others, the effects are minimal. For instance, ingestion of plants (Senecio spp.) which contain pyrrolizidine alkaloids cause severe liver disease in horses, to a lesser extent in cattle, and only modest effects in sheep and goats (Jubb, 1970). Horses are also highly susceptible to toxins in the yellow-star thistle (*Centaurea solstitialis*) leading to Parkinson's Disease like symptoms. To date, there are no other species that have been found to be susceptible to these toxins (Kingsbury, 1980). Thus the most appropriate animal for developing diagnostic tests and treatment regimes is the horse (Bull, 1968).

Because toxicokinetic studies involve absorption from the site of administration, distribution, metabolism, and elimination of the chemicals or their metabolites via different excretory routes, these studies must be conducted in intact animals. For instance, the efficacy of antibiotics in controlling urinary tract infections caused by bacteria in dogs must be carried out in this species before it can be determined safe and effective for use in clinical cases. In addition, manifestation of desirable and undesirable effects in response to chemicals usually involves some degree of alterations in physiological functions of the whole animal and, therefore, it is again unavoidable not to use animals in such studies. Alterations in the physiological functions of the whole animal can not be duplicated in tissue culture or computer models at present.

Continued but Modified Use of Whole Animals. Some reduction in numbers will be brought about by screening of new toxins and chemicals in *in vitro* systems. This will mainly be for substances that would not get past the acute/subacute toxicity screening.

Alteration of pain is carried out with analgesic under circumstances where it is apparent that animals are suffering. In chronic toxicity/carcinogenicity tests, euthanasia of animals reaching a preselected endpoint (e.g., obvious malignant tumor development) can be considered. Substitution of lower mammals (e.g., laboratory rodents) is already being practiced where possible. Many initial studies are carried out in these species before determining whether the substances are toxic in domestic species. Since there are important interspecies differences in the action of drugs and chemicals, study (or at least validation) is needed in the animal species of concern to veterinary medicine.

Lower vertebrates and non-vertebrates have not been used to any extent in toxicological studies in veterinary medicine.

Use of Animal Components. There are many instances where the techniques of molecular and cellular biology are being used to promote the understanding of the biochemical mechanisms of toxicity. These techniques include: 1) use of hepatocytes and pneumonocytes in tissue culture systems to evaluate effects of toxins on these target cells; 2) organ culture; and 3) organ slices for studying metabolic alterations caused by various toxic compounds (Plaa, 1980). There is no doubt that such studies will continue to contribute to our understanding of the effects of chemicals at the molecular level.

In development of new products, *in vivo* tests are increasingly important in screening and in scaling the relative potency/toxicity/carcinogenicity of substances. Chemical products (drugs, pesticides, etc.) being seriously considered for production or which are by-products reaching the environment must be tested in animals. Tests in living animals are essential for final safety evaluation and, particularly, for risk assessment.

Use of Non-living Systems. Toxicity always refers to harmful actions of chemicals on biologic tissues (Loomis, 1978). Therefore, toxicants have to interact with some cellular components of the living tissues in order to elicit a toxicologic response. For example: 1) Amygdaline, a cyanogenic glycoside produces its lethal effect by tying up the electron transport in the cytochrome A-A3 complex of the mitochondria (Smith, 1975); 2) gentamycin, an antibiotic, produces acute renal damage typical of alterations of proximal tubular function (Appel, 1977) by interacting directly with the renal tissue; 3) toxic nature of mercury has been recognized since antiquity; mercurial compounds produce nephrotoxicity by acting directly on the renal tubular cells, and 4) a series of compounds including organophosphorus acrylamide, hexanedine cause peripheral axonopathies by acting directly on the peripheral motor nerves (Norton, 1980). Non-living systems are not used in toxicologic research.

Use of Computer Programs. Programs are useful for mathematical modeling and construction of 3-dimensional matrices linking results of various *in vitro* tests (toxicity, mutagenicity, etc.) with physicochemical characteristics and with *in vivo* endpoints. The goal is to make predictions on relative potency/toxicity of new substance by scaling against benchmark substances. Final evaluation of new toxicants/ chemicals, however, must be in living animals.

Virology

Statement: Virology is the study of structural aspects of viruses as well as the biological activity of viruses within the host system(s).

Continued Use of Animals. There is a definite requirement to continue to study viruses in domestic animals. The need to continue studies includes the fact that some viruses such as the adenomatosis virus of sheep are capable of replicating only in the host of origin (Gillespie, 1981). Hog cholera virus, visna virus of sheep, equine infectious anemia virus, canine parvovirus, etc. appear to be species specific and as a result the effects of these viruses on animals can only be evaluated only in the host species (Gillespie, 1981). The biologic activity of viruses can only be assessed by studying their effects on the host system. For instance, bluetongue virus infection in sheep causes disease by damage to small blood vessels leading to microinfarction whereas bluetongue disease in cattle is an immunologically mediated hypersensitivity disease (Mahrt, 1984; Stott, 1982).

Continued use of animals is necessary when new virus isolates are made from sick animals. It is important to isolate and characterize the virus, and inoculate it back into other experimental animals of the same species to prove that the new virus is capable of producing the disease (Gillespie, 1981).

With some diseases such as simian acquired immune deficiency syndrome (SAIDS), the complex expression of a variety of other infectious diseases in animals masked the effects of the SAIDS agent until the virus was experimentally inoculated and followed in the rhesus monkeys (Graven, 1984; Marx, 1984). It was only through these studies that it has been possible to show that the virus causes immunosuppression.

Continued but Modified Use of Whole Animals. Once a virus has been shown to cause disease, every effort is made to grow the virus in organ or cell cultures. Much of the present day virology is being carried out in organ cultures obtained from animal tissues from abattoirs or in established cell culture lines. These systems provide a more refined medium for characterizing the structure and molecular aspects of viruses as well as viral cytopathology.

Pain is usually only of concern when the biological effects of viruses are studied in animals. In some instances pain is part of the effects of the virus; however, viruses commonly cause depression (Gillespie, 1981). Animals that are suffering undue pain are euthanatized.

Lower mammals are often used for experimental purposes if the particular virus will replicate in their tissues. The pathogenic properties of bluetongue virus in the developing nervous system can be studied to some extent in mice and hamsters rather than fetal calves and lambs (Narayan, 1972; Richards, 1967). When an animal model is available, it is used. Not all animal viruses will grow in lower mammals (Gillespie, 1981).

Lower vertebrates and non-vertebrates are not commonly used as substitutes for higher mammals because organ and cell culture systems are more effective. Some veterinary researchers study viral diseases such as channel catfish virus disease, herpesvirus disease of salmonids, infectious pancreatic necrosis disease, in lower vertebrates such as fish and snakes (Roberts, 1978).

Use of Animal Components. Virology researchers have pioneered the use of organ and cell culture systems for propagating and studying viruses. Much of veterinary virology is carried out in organ cultures, primary cell cultures obtained from abattoir animals, or on continuous cell lines. This has greatly reduced the use of animals for propagating virus in veterinary research.

Use of Non-living Systems. At present it is not possible to utilize chemical or physical systems to completely replace biologic systems (i.e., cell cultures) in veterinary virology. As a better understanding of the genetics of viruses evolves, it will be possible to synthesize portions of virus subunit proteins.

Use of Computer Programs. Computers are being used to assist in defining the genetic sequence of viruses in veterinary medicine. Genetic material obtained from cells is purified and analyzed in a sequencator. In order to record and analyze all of the DNA or RNA sequnces, computers and computer programs are used (Staden, 1980). Other than this, the use of simulated models is somewhat limited at this time.

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